OPTIMISATION OF BIOGAS PRODUCTION FROM PERCOLATING PACKED BED ANAEROBIC DIGESTERS

by

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ABSTRACT

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Percolating packed bed digesters were operated successfully in a batch mode using a wheat straw - dairy manure substrate of between 21 and 27% total solids. The vessels used had a nominal 10 l volume and were constructed of perspex. Temperatures of 25-35°C were used, recirculation rates of 0-15 litres.hr^{-1} (the digesters having a diameter of 0.18 metres thus corresponding to superficial flow rates of between 0 and 0.382 $l/m^2/hr$), solid : liquid ratios of 1:1 to 4:1 and bed heights of 0.26 to 2.05 metres.

The optima found were a temperature of 35°C, recirculation rate of 3 litres.hr^{-1}, a solid : liquid ratio of 2:1 and a bed height of 1.5 metres. Experiments were conducted for periods of up to 70 days, though operation beyond a 40 day period was found to produce little extra biogas. The performance compared favourably with other high solids waste digesters with gas yields of 0.305 m^3/kg VS added and solids losses of 47% volatile solids and 64% cellulose being obtained over a 40 day period. No major problems of inhibition or blocking occurred.

Linking of digesters in series via their recirculation systems was found to be advantageous. Gas yields were found to be increased by approximately 18% and solids losses increased by approximately 20% when the waste was treated in this semicontinuous manner. These increases were found to be a result of the rapid transfer of well-adapted bacteria to the fresh digester. Lag phase in the fresh digester was reduced by three days and potentially inhibitory levels of volatile fatty acids were not present. Concentrations of up to around 5000 ppm VFAs were found during the start-up of batch digesters causing some inhibition of gas production. During semi-continuous operation however concentrations of around 2000 ppm were developed when fresh digesters were linked in, no inhibition occurred and in fact this concentration proved stimulatory to gas production. Experimentation into the optimum retention time of a maximum of three digesters in series was conducted, with retention times of 90, 60 and 30 days being considered. A 30 day retention period was found to depress gas production due to unstable conditions when fresh digesters were added by up to 32% compared with Batch Operation. Gas production was increased at both 60 and 90 day retention times by amounts similar to those previously stated. A retention time of 60 days was found to be optimum as little extra gas was produced after this time, with volatile solids losses being increased by only 9.3% by operating for a further 30 days.

Colonisation of the solid substrate was shown to be rapid, by the use of adenosine 5'triphosphate analysis, gas production rate and electron microscope analysis. In addition a dynamic bacterial population appeared to be present in the solid phase with the rates of growth and attachment being approximately equal to the rates of decay and detachment. When digesters were operating in their steady phase, methanogens were present in the liquor at concentrations of between 10^6 - 10^7/ml and non-methanogens at between 10^7- 10^8/ml showing a large population of bacteria to be present for the inoculation of fresh digesters.

CONTENTS

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CHAPTER 1

INTRODUCTION

In anaerobic environments the ultimate fate of the complete microbial dissimilation of complex organic compounds such as carbohydrates, proteins and fats is the formation of methane and carbon dioxide (Mah *et al*, 1977). This occurs in a range of natural environments where the level of molecular oxygen is extremely low, alternative electron acceptors are absent and organic polymers are found. These include lake and marine sediments, muds, decaying trees, animal guts (particularly those of ruminants) and hot springs. In most of these environments the substrates for methanogenesis (hydrogen, carbon dioxide, acetic acid and some other low molecular weight compounds) are formed by the microbial degradation of complex organic compounds by consortia of bacteria.

This naturally occuring phenomenon can be exploited as a method of waste treatment and energy production in a controlled environment, and is termed anaerobic digestion.

1.1 The Biochemistry and Microbiology of Anaerobic Digestion

Most wastes treated by anaerobic digestion are a complex mixture of carbohydrates, fats and proteins, and the reactions by which these are converted to methane and carbon dioxide are even today not fully understood. Indeed for many years *Methanobacillus omelianskii* was described as a strictly anaerobic bacterium which

obtained its energy for growth by oxidizing ethanol to acetate, but was shown 40 years later to be in fact two bacterial species, one of which ferments ethanol to H_2 and acetic acid, and the second utilising H_2 for growth and methane production (Bryant et al, 1967)

The digestion process was initially considered to consist of two distinct stages:- (i) an initial hydrolytic and fermentative stage leading to the production of volatile fatty acids, organic acids such as succinate and lactate, aldehydes, methanol and other higher alcohols and gases such as hydrogen and carbon dioxide.(ii) a methanogenic stage which results in the conversion of stage (i) products to methane and carbon dioxide. Studies since the late 1960's have shown however that methanogenic bacteria are only able to utilise acetate, formate, methanol, hydrogen, carbon dioxide and a few other one-carbon compounds as substrates for methane production (see for example Balch et al, 1979) Subsequent research showed there to be two additional groups:- (i) hydrogen producing (or proton reducing) acetogenic bacteria responsible for the interconversion and breakdown of fermentation products to acetate, hydrogen, carbon dioxide and a variety of one carbon compounds (for a review see Wolin & Miller, 1982). (ii) Homoacetogenic bacteria which convert hydrogen, carbon dioxide and a variety of one and multi-carbon compounds to acetic acid alone. Thus the anaerobic digestion process is effected by the integrated action of at least four trophic groups of bacteria (Fig.1.1) with clearly defined and distinct carbon metabolising functions (Zeikus, 1980). In addition the methanogenic bacteria can roughly be described in two groups, 'Acetoclastic' methane bacteria (e.g. Methanosarcina barkeri) which utilise predominantly acetic acid; and hydrogen

utilising methane bacteria (e.g. Methanospirillum hungatei) which utilise predominatly hydrogen and carbon dioxide (Mosey 1983).

Over the last decade the significance of hydrogen partial pressure has become widely recognised to be of considerable importance to the anaerobic digestion process (Verstrate, 1983) and a subtle form of control operates through the hydrogen utilising methane bacteria. Hydrogen producing acetogenic bacteria will only function at low hydrogen partial pressures, which are maintained by the hydrogen utilising methanogenic bacteria. Thus methane bacteria can regulate the internal metabolism of the acid forming bacteria (Boone & Bryant, 1980).

When the system is functioning smoothly, the methyl group of acetic acid is the precursor for around 70% of the methane formed in anaerobic environments (Jeris and McCarty, 1965; Smith and Mah, 1966) according to the following simplified equation;

$$CH_3COO^- + H^+ \longrightarrow CH_4 + CO_2$$

the remainder being produced from hydrogen and carbon dioxide;

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$

Methanogenesis is in reality an extremely complex sequence of reactions, incorporating numerous cofactors and coenzymes which is still being elucidated. Vogels and Visser (1983) have shown methane to be released from a sequence of co-factors by a methyl reductase system requiring ATP and magnesium ions. This may not be the whole story however as under certain conditions there is no requirement for ATP in methanogenesis.

1.1.1 The Methanogenic Bacteria

Methanogens are believed to be part of an ancient group of bacteria known as the 'Archaebacteria' (Fox et al, 1977, Balch et al 1979) along with other highly diverse and specialised bacteria such as halophiles and thermoacidophiles and are characterised by their ability to exist in hostile environments. For example, Methanobacterium thermoautotrophicum is capable of producing methane from hydrogen and carbon dioxide at 80°C (Zeikus and Wolfe, 1972). It is possible that all Archaebacteria have a common evolutionary origin and their similarity with Eubacteria is due to convergent evolution.

Methanogenic bacteria have many unique properties, the most notable of which is their cell wall structure which contains no diaminopimelic acid or muramic acid (peptidoglycan) (Kandler and Hippe, 1977), there is also great variation within Archaebacterial cell walls, four distinct types having been found (Kandler and König, 1978; König and Kandler, 1979). Methanogens also have characteristic ribosomal and transfer RNA (Wolfe, 1979). The lipids of Archaebacteria are mainly non-saponifiable having ether instead of ester bonds and contain large amounts of squalene (Wolfe 1982). Archaebacteria, and methanogens in particular, have a wide range of unique co-factors and co-enzymes (Weose et al, 1978) such as co-enzyme M (CoM) a simple sulphonic acid, whose thiol group reacts to form methyl-CoM. Co-factor F_{420}, an unusual flavin acts as an electron carrier. In addition there are co-factors F_{430} and F_{342} and others are currently being characterised, such as tetra- hydromethanopterin (THMP) elucidated by Vogels and Visser

(1983). Methanogens are thought to have no cytochrome or quinones involved in electron transport and phosphorylation, and have typical genome sizes less than those of Eubacter such as E.Coli (Klein and Schnoor, 1984), though there is some evidence of plasmid material being present (Thomm et al, 1983).

Methanogens are thus a highly specialised and yet diverse group of bacteria, having many unique properties, and occupying a unique place in the carbon cycle.

Many other types of bacteria have been isolated from anaerobic digesters, and include obligate anaerobes such as Clostridia and facultative anaerobes such as Propionobacter, Butyrobacter and Lactobacillus.

Other bacteria not directly involved in methane production are also present in anaerobic digesters. The most common of which are probably sulphate reducing bacteria such as Desulphovibrio.

However of greater interest to this particular study are the cellulolytic bacteria and their action which is considered in the following section.

1.1.2 Cellulose and Cellulolytic Bacteria

Studying the mass balance of the major stages of anaerobic digestion (hydrolysis, acid formation and methanogenesis) Van Velsen (1977) concluded that hydrolysis of polymeric substrates such as cellulose is the rate limiting step in digesters with retention times greater than 15 days.

Cellulose is a major constituent of plant cell walls, and is

the most abundant organic material on earth. It is a polymeric carbohydrate composed of unbranched chains of anhydroglucose linked by B(1-4) glucosidic bonds. However cellulose does not occur in its pure form in biological materials and is associated with a variety of other polysaccharides such as starch, pectin and hemicelluloses. Hemicelluloses vary in composition, and are polymers of galactose, mannose, xylose, arabinose and other sugars. Lignin may also be associated with cellulose, and tends to limit the extent to which cellulose can be degraded.

Cellulose in plant cell walls forms microfibrils 50 - 400 angstroms in width (Wood, 1970) and the cellulose molecules are found in parallel arrays that are probably the result of regular folding of individual molecules. Microfibrils contain regions of both amorphous and crystalline cellulose which differ in their susceptibility to degradation, the crystalline arrangement consisting of ordered arrays of parallel strands of cellulose which is relatively resistant to degradation.

Anaerobic cellulolytic bacteria are responsible for most cellulose degradation in anaerobic digesters. These include Clostridium thermocellum, Ruminococcus albus, Ruminococcus flavefaciens, Bacteroides succinogens and Bacteroides acetogens (Groleau and Forsberg, 1981; Ait et al, 1979). Other cellulolytic anaerobes include Acetivibrio cellulolyticus (Patel et al, 1980), Clostridium cellobioparum (Hungate, 1954) and Clostridium polysaccharolyticum (van Gylswyk, 1980).

Cellulose is degraded by a complex of enzymes comprising three major components; Endoglucanases which hydrolyse bonds between glucose residues, Exoglucanases which act at the

non-reducing ends of cellulose molecules and finally Cellobiase which cleaves cellobiose and cellodextrins to give cellulose. Not all cellulolytic organisms exhibit all of these actions.

The enzymic attack on cellulose is probably initiated by Endoglucanases, Exoglucanases are then able to remove cellobiose or glucose from the cleaved cellulose molecules when Cellobiase cleaves the cellobiose produced into glucose.

The location of bacterial cellulolytic enzymes varies, for example in Clostridium thermocellum only exocellular glucanases have been detected, while other species produce only cell bound glucanases and other produce both forms of enzyme. All bacterial cellobiases are thought to be intracellular.

As the waste used in this study contains large amounts of cellulose, cellulolytic bacteria and the enzymes they produce are of great importance.

1.2 The History of Anaerobic Digestion

The technology of anaerobic digestion has existed for around 100 years (McCarty, 1982), when human sewage was treated in unheated and unmixed septic tanks, and the gas produced rarely utilised. During the early part of the 20th century sewage digesters were refined with the introduction of heating and longer solids retention times and anaerobic digestion became increasingly important in the treatment of sewage. Since 1950 high rate digesters incorporating heating and mixing were developed (McCarty, 1982) to treat sewage, and the gas produced used to heat and light the sewage works and to help power aerobic treatment facilities

(Hobson, 1982).

The anaerobic digestion of wastes other than sewage is relatively recent, except during the second world war when methane was used as a substitute vehicle fuel (Hobson, 1982). The use of biogas to drive converted petrol and diesel engines is today being reconsidered (Anon, 1981). Many wastes are now being used as a source of feedstock for anaerobic digestion, though some are still in the experimental stages. These include food processing wastes (Landine et al, 1983; Silvero et al, 1980), chemical industry wastes (Chou et al, 1978; Frostell, 1982), distillery wastes, (Das et al, 1983), municipal solid wastes (Farooq et al, 1983) and agricultural wastes (Hashimoto, 1981; Hashimoto, 1984; Hawkes and Young, 1980; Hobson, 1981). The growth of "energy crops" specifically for the production of energy is now also being considered (Carruthers, 1982; Samson and LeDuy, 1982; Stewart, 1977).

Much of the current interest in anaerobic digestion as an alternative fuel source owes its origin to dramatic increases in the price of fossil fuels (particularly oil and petroleum) in the 1970s, and the realisation that fossil fuels are of a finite nature. Energy from biomass is thus considered attractive due to its renewable nature, and estimates suggest terrestrial biomass productivity in the region of 125×10^9 tonnes dry matter per year (Box, 1975) in addition to this aquatic environments could yield significant quantities of biomass for energy production (Risser, 1981).

The anaerobic digestion of agricultural wastes has been practised in the third world for many years, where labour costs are

low and conventional fuels very expensive, thus small, simple and relatively inefficient digesters are economically viable and can markedly improve the lifestyle of the villagers. This technology has been practised for many years in China (Wang et al, 1980), Korea, where 27000 small digesters have been built since 1969 and in India, which has over 100,000 small digesters (Jain et al, 1983). In industrialised countries the economics of the anaerobic digestion of agricultural wastes is different, and digesters need in general to be larger and more sophisticated to be economically viable. It is probable that using existing digester technology, anaerobic digestion on small farms will only become viable when pollution control becomes a greater issue, and the economics are not viewed purely in terms of biogas production. There is though a trend over the last 15-20 years to move animal production into large intensive farming units, where cattle, pigs and fowl are reared in large numbers undercover all the year round. In the case of cattle this may be as much as 10,000 head (Ashare et al, 1979). This type of unit will often have little associated land for waste disposal, and due to the offensive nature of the waste and high treatment charges from local authorities be required to treat the waste on site.

Many of the wastes produced on farms differ physically and chemically from sewage, and thus cannot be effectively treated in conventional 'High Rate' digesters. In addition, the present economics of the anaerobic digestion of farm waste is based on gas production rather than pollution control. Many alternative digester designs have been developed (Callander and Barford, 1983b) in an attempt to minimise capital and running costs, and will be

considered later.

1.3 The Benefits of Anaerobic Digestion

Anaerobic digestion gives rise to a reduction in the organic biodegradable fraction of the waste. The resulting solids have a lower bulk, are more "settleable" and thus make further treatment easier (Hobson et al, 1981). Anaerobic digestion lowers the offensive odours emanating from the waste, primarily due to a reduction in the concentration of low molecular weight organic acids.

There is a strong reduction in pathogenic bacterial counts. (Stuckey, 1983)

The effluent of digesters may be used as a fertilizer, the majority of the influent nutritional value (nitrogen, phosphates, potassium and trace elements) being maintained. Indeed there is usually an improvement in fertiliser value after digestion since these elements are transformed into more readily available forms (Marchaim, 1983).

The methane produced can be used for heating, lighting or to produce electricity via an engine and generator (Marcamba et al, 1983)

1.4 Types of Digesters

In conventional "High Rate" digesters, (Fig.1.2) the contents are completely mixed, giving the various feedstock components the same retention time. In a heterogenous waste (such

as cattle slurry) the solids are digested more slowly than soluble substrates and bacteria present may form close associations with the solid particles. In addition methanogenic bacteria have long doubling times being in terms of days rather than hours as for most Eubacteria and thus the retention time must be long enough to prevent their washout. The size of a high rate digester is primarily a function of feed volume and retention time (Hashimoto and Chen, 1980) and thus long retention times will lead to large digesters. If the solids can be retained or recycled, smaller and cheaper digesters are feasible, or if the bacteria themselves can be retained, digestion can take place independently of bacterial growth rates allowing greater throughput of the waste into smaller digesters (Callander & Barford, 1983a). These principles underly many of the new trends in anaerobic digester design, which can be considered as second generation reactors.

The anaerobic contact process (Dimovski, 1980; Lane, 1984) allows effluent from a continuously stirred tank reactor (CSTR) to pass into a settling tank, where the solids are seprated and returned to the reactor (Fig.1.3). Using this method biogas production rates of up to $3m^3$ per m^3 reactor per day are possible (van den Berg, 1982).

With wastes containing low levels of particulate solids, fixed film or filter digesters can be used successfully (van den Berg & Kennedy, 1983). In this type of digester bacteria are attached to an inert matrix, over which the influent is passed either in an upflow or downflow manner (Fig.1.4). Many types of support matrix (or packing material) have been used, for example gravel, plastic rings and spheres, fired clay and ceramics

(Colleran et al, 1982). This type of filter digestion is at present being used to treat certain wastes commercially (Szendrey, 1983).

Bacteria can also be retained in the digester by attachment to particles (usually sand). In the Attached Film Expanded Bed process (Fig. 1.5) the sand particles give the bacteria a high settling velocity, and by pumping the influent upwards at high rates, they remain suspended, and high rates of mass transfer are obtained (van den Berg, 1982; Bull et al, 1984)

The Upflow Anaerobic Sludge Blanket (UASB, Fig.1.6) operates by a similar principle, but contains no support medium. Instead the bacteria present flocculate and form clumps which have a high enough settling velocity to avoid washout (Lettinga et al, 1983; Hall et al, 1982). Mixing in the digester is accomplished by the agitation caused when gas is produced.

Plug flow digesters (Fig.1.) are elongated reactors through which the waste moves by displacement. The flow of waste does not resemble true "plug flow" due to mixing caused by gas production. Plug flow digesters may be of a horizontal or inclined design (Hawkes et al, 1981), and can in their simplest form be cheaply constructed and have low operational costs (Jewell et al, 1981) though they are highly susceptible to severe scum formation.

It has been suggested that no single set of conditions is optimal for all the reactions involved in the anaerobic digestion process, and that the use of sequential reactors operating under different conditions of for example pH and retention time may improve the efficiency of the process. Ghosh and Klass (1978) have suggested that the pH optima is lower in the hydrolytic than the

methanogenic stage. There are numerous examples of two phase digestion systems (Verrier et al, 1983; Ghosh et al, 1983). In many cases solid wastes such as vegetable tops are liquified in the first reactor giving rise to a high strength effluent containing high levels of volatile fatty acids. This effluent is then treated in a methanogenic reactor usually of the second generation type such as the UASB (Rijkens, 1981). Many of the process advantages of this type of digestion could be offset by the increased capital and running costs of having two or more digesters treating one waste.

1.5 The Anaerobic Digestion of Solid and Semi-Solid Wastes

The strategies for anaerobic digestion described above, with the exception of two phase digestion are intended for the digestion of low solids or soluble wastes. Many organic wastes which could be treated anaerobically are obtained in a solid or semi-solid form. This type of waste also occurs in large quantities, for example though most animal wastes are produced as a slurry, the housing methods and bedding used leads to a material of much higher dry matter content. Cattle bedded on straw gives rise to a waste of 20% total solids or more. In France alone, where this practice is common, it could theoretically account for 80% of available biogas energy, equivalent to 5.4 million tonnes of fuel oil per year (Finck and Goma, 1982). Perhaps the largest single source of high solids waste suitable for anaerobic digestion is the putrescible fraction of household waste, or Municipal Solid Waste (MSW). There are numerous examples of the treatment of MSW (Wise

et al 1978, Leeper et al, 1982) many of which use a two-phase process, and all require some form of pretreatment before digestion can commence. A second form of digestion, not applicable to other wastes is available for MSW, namely Landfill (Zimmerman et al, 1983). Here gas produced naturally in municipal waste tips is harvested and utilised. There are many example of landfill sites producing utilisable biogas. These include sites in the United States (Walter, 1983) where a site in Florida accepting 100 tons/day of refuse is producing over 700,000 ft^3 of gas per day, and in Europe where London Brick Land- fill Ltd. has a partially filled site containing $^3/_4$ million tonnes of domestic refuse allowing 8.5m^3/minute of biogas at 60% methane to be pumped off (Richards, 1981).

The anaerobic digestion of high solids waste, or "dry" anaerobic digestion, that is to say wastes having a total solids content of over 15%, has numerous advantages. They occur frequently and in large quantities. To be treated conventionally they require dilution to between 5 and 10% total solids, the usual range of operation for conventional digesters (Mills et al, 1979). This could lead to as much as a five fold aqueous dilution before digestion can take place (Hall et al, 1985a), increasing the unit cost of treatment due to an increased heating requirement, digester volume and post-digestion treatment facilities (Bridgewater, 1977a and b; Kottowitz and Schulte, 1982). In addition, conventional digesters are susceptible to mechanical failure when fibrous matter is present.

Pretreatment would thus be required to reduce the size of any fibrous material, or remove it completely again leading to

increase in both capital and running costs. Further it has generally been accepted that the rate limiting step in the anaerobic digestion of soluble monomeric substrates is the methanation of volatile fatty acids (Hobson, 1974), whereas for polymeric biomass substrates hydrolysis is the limiting step (Ghosh and Klass, 1978). Thus a longer retention time may be required for these substrates, increasing digester volume and cost if they are to be treated conventionally. Operational difficulties such as scum formation (Buswell, 1947) may also be avoided by the direct treatment of solid waste, and equipment at present in use on farms producing this type of waste could be used in the operation of solid waste digesters (Wujcik and Jewell, 1980).

As with most wastes, not all the dry matter content of the waste is biodegradable, a percentage of it will be ash. In addition naturally occurring organic wastes particularly in those containing plant material, a portion of the volatile matter will be of a lignaceous nature, and lignin masked cellulosic material. It is assumed that lignin plus an equal percentage of cellulose is non-biodegradable, based on the observation that peat is approximately 50:50 lignin:cellulose. Recent studies however (Colberg and Young, 1982) have shown that soluble fragments of lignin can be broken down under anaerobic conditions, it is not clear whether this will be significant within the digester. To improve the biodegradability of such substrates, pretreatments both mechanical (eg. ball milling) and chemical (eg. alkali washing) can be used to increase biogas yields (Baccay and Hashimoto, 1984); McCarty et al, 1978; Schurz, 1977).

In the United Kingdom agricultural wastes have a total

energy content equivalent to 4.2% of primary fuel consumption. Of this cattle waste accounts for 4.6×10^7 tonnes fresh weight and straw 1.2×10^7 tonnes fresh weight (Larkin, 1982), and therefore represents a considerable potential source of energy via anaerobic digestion. As the total solids content of the feedstock is one of the most important factors governing net energy production from anaerobic digesters (Hawkes, 1980; Bousfield et al, 1979) it is clearly of economic interest to digest this type of waste in the form in which it is produced.

1.6 Studies on the Anaerobic Digestion of High Solids Agricultural Wastes

A number of investigations have been conducted into the anaerobic digestion of solid and semi-solid agricultural wastes. One of the first was that of Wong-Chong (1975). Batch experiments were conducted using dairy manure of 20.8% total solids, showing this mode of digestion to be feasible, by producing $11.3ft^3$ biogas per lb volatile solids (VS) destroyed with a 36.3% VS destruction over a 140 day period.

Several studies have been conducted into the anaerobic digestion of mixtures of manure and plant matter (Laura and Idnami, 1971; Robbins et al, 1979). All have shown greater methane production from the digestion of mixtures than from an equivalent amount of manure alone. Hills (1979) using a cattle manure and straw mixture showed the importance of non-lignin Carbon : Nitrogen ratio, cattle manure alone having a C:N ratio of around 8 : 1. Hills (1979) found an optimum C:N ratio of 25-30 : 1, and further work by Robbins et al (1983) gave an optimum of 32 : 1.

Wujcik and Jewell (1980) used a 25% total solids mixture of dairy manure and wheat straw in 4.5l bench scale reactors, and obtained gas yields of 0.2m^3 biogas/kg VS added at a loading rate of 7.86gVS/litre reactor per day. A 4550 litre pilot scale reactor was also operated on the same waste and gave comparable results. It was also shown that above 30% total solids (TS) gas production rates are depressed with increasing TS. Further studies (Jewell et al, 1981) on dry anaerobic fermentation have concluded that this mode of digestion could be highly competitive with existing anaerobic digestion systems and that process control was possible.

Hills (1980a) conducted investigations into the digestion of dairy manure at high solids concentrations using a 70 litre batch fed digester. Total solids concentrations ranged between 17.9 and 20.3% and at a retention time of 15 days obtained a gas yield of 0.18m^3/kg VS added. Further studies (Hills 1980b) using a combination of dairy manure and barley straw of 17.1% total solids obtained gas yield of 0.19m^3/kg VS added at a 15 day retention time. Hills and Roberts (1980) used dairy manure and various crop residues (barley straw, rice straw and rice hulls) showed the optimum carbon : nitrogen ratio to be between 25 and 32:1 giving a maximum volatile solids reduction of 34.4% and a gas yield of 0.37$m^3 CH_4$/kg COD destroyed.

A number of novel digester designs have been produced to operate on high solids waste in recent years. For example (Himmel and Lafferty) used a 50m^3 digester consisting of concentric cylinders to operate on a cattle manure/straw substrate of 13.3% TS. Numerous problems such as scum formation and blockages were encountered, but in periods of stable operation gas yields of

$0.32m^3/Kg$ VS added were obtained at a residence time of 42 days. Finck and Goma (1982) developed a system to operate on semi-solid waste containing fibrous matter which was based on the flotation of these fibers. Using waste of 14% TS at a 17.4 day retention time, gas yields of $0.274m^3/kg$ VS added were obtained. Kottowitz and Schulte (1982) used a rotating drum to mix high solids beef manure of 26% total solids and produced $0.30m^3$ biogas/kg VS added at a solids retentions time of 53 days.

Due to the special problems associated with the anaerobic treatment of high solids waste especially those containing fibrous matter, digesters having few moving parts in contact with the waste could be highly advantageous, in reducing the frequency of blockages and hence digester down time which will significantly affect the economics of the process. To this end Goldberg et al (1981) designed the 'biofunnel' capable of being continually loaded with a 21% TS mixture of pig manure and straw, though results showing the efficiency of the process do not appear to be available.

These new digester designs all involve moving parts to a greater or lesser extent which may put them at a significant disadvantage. Petersen (1981) considered the batchwise digestion of fixed beds packed with a manure-straw mixture of 19% total solids. Gas production was enhanced using a liquor recirculation system; $0.16m^3/kg$ VS added being obtained after 39 days without recirculation and $0.24m^3/kg$ VS added being obtained after 41 days with liquor recirculation. The advantages of this system and that described in the following chapters is that the only moving part is the liquor recirculation pump, the liquor containing very low

levels of solid material (approximately 2% dry matter).

The effects of scale up on high solids digesters have not been fully investigated, and it is not known how factors such as compaction will affect them.

Figure 1.1. Bacterial Groups Involved in the Anaerobic Digestion Process

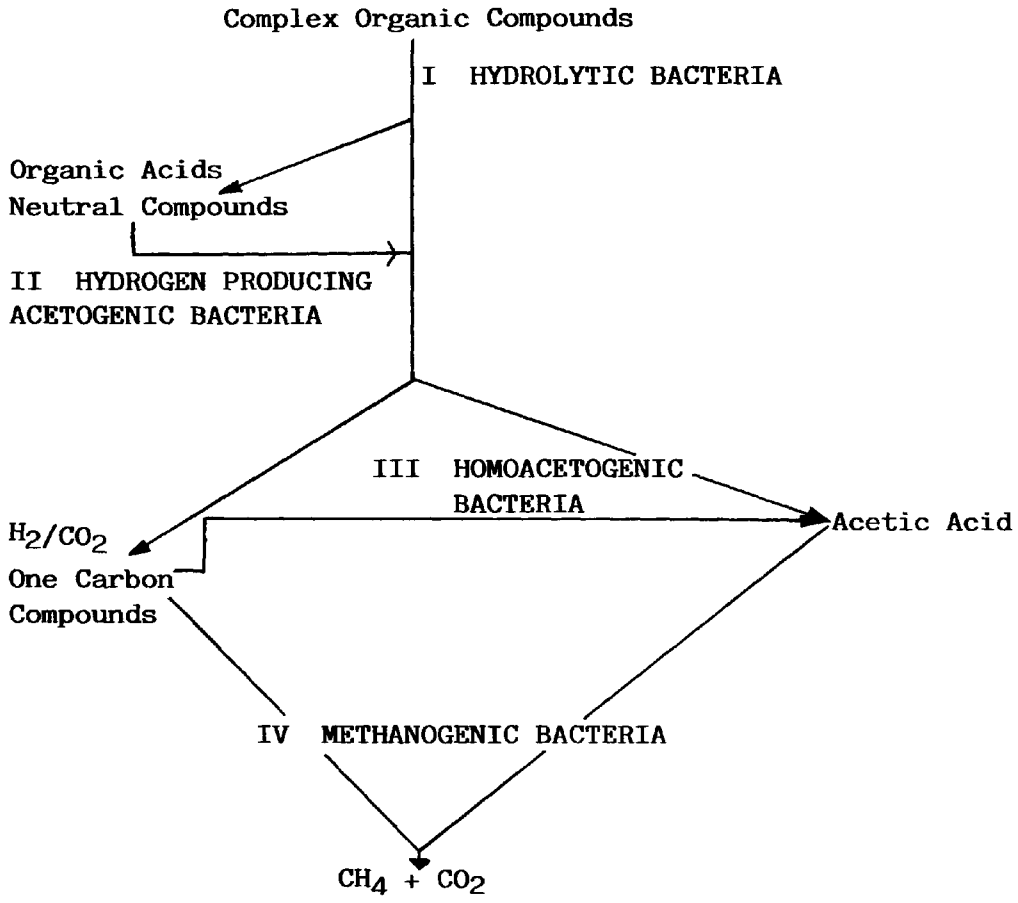


Figure 1.2. High Rate Digester

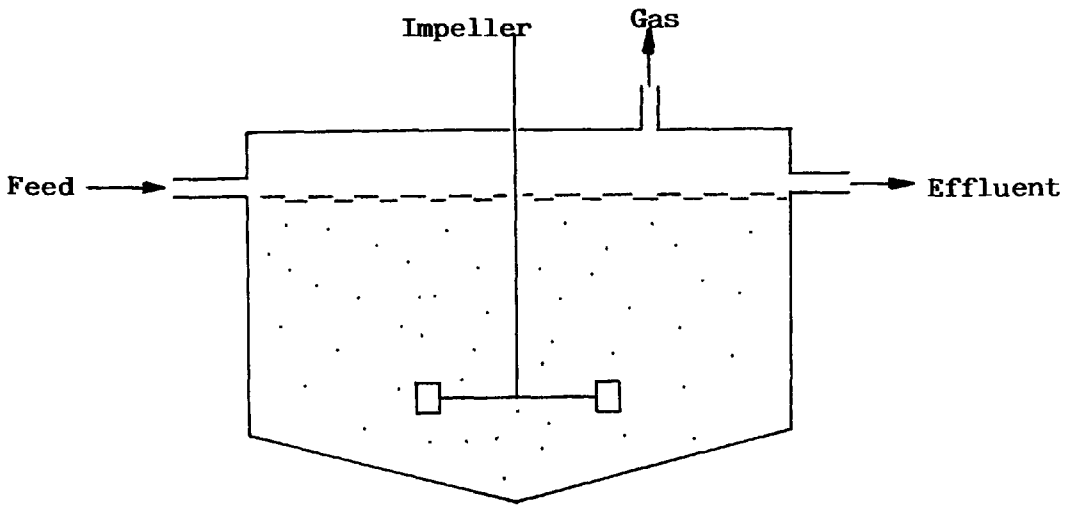


Figure 1.3. Anaerobic Contact Process

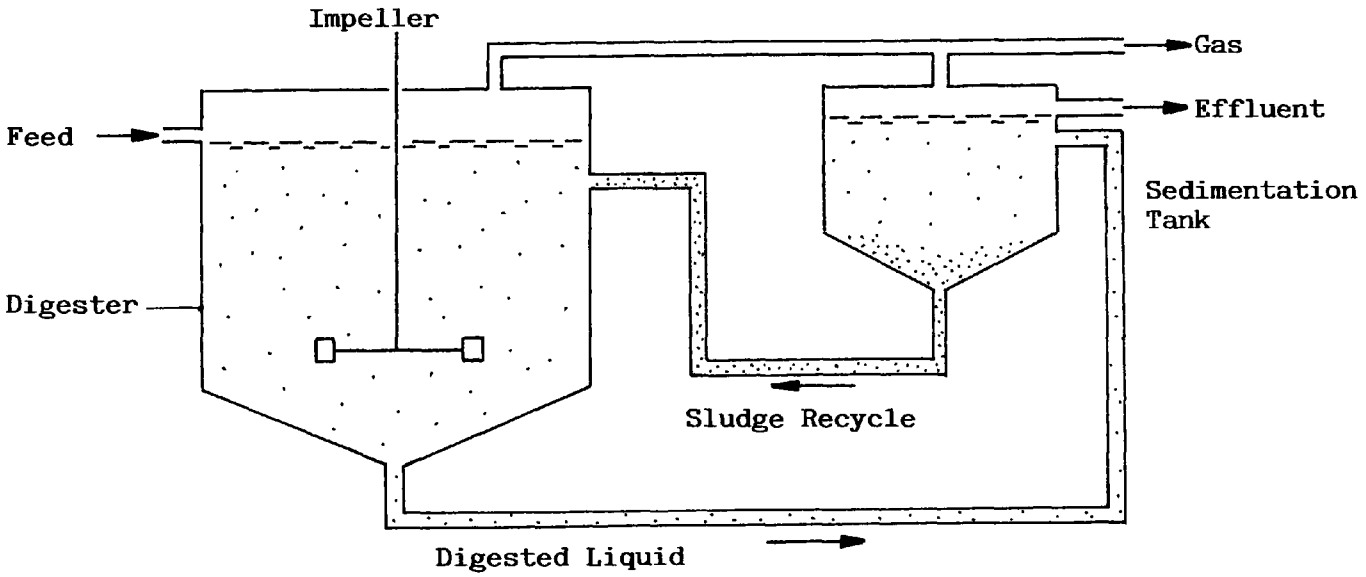


Figure 1.4. Anaerobic Filter

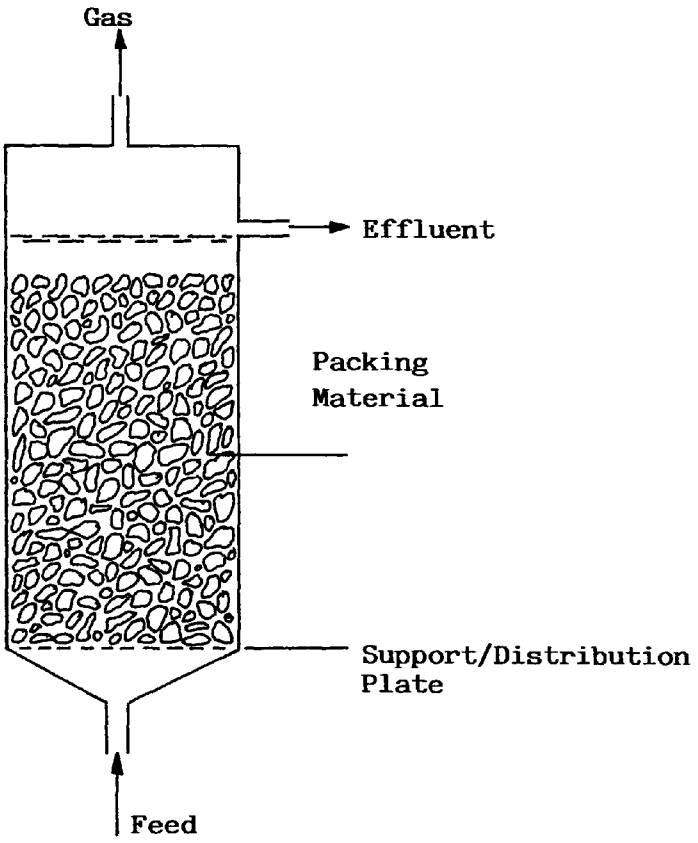


Figure 1.5. Attached Film Expanded Bed

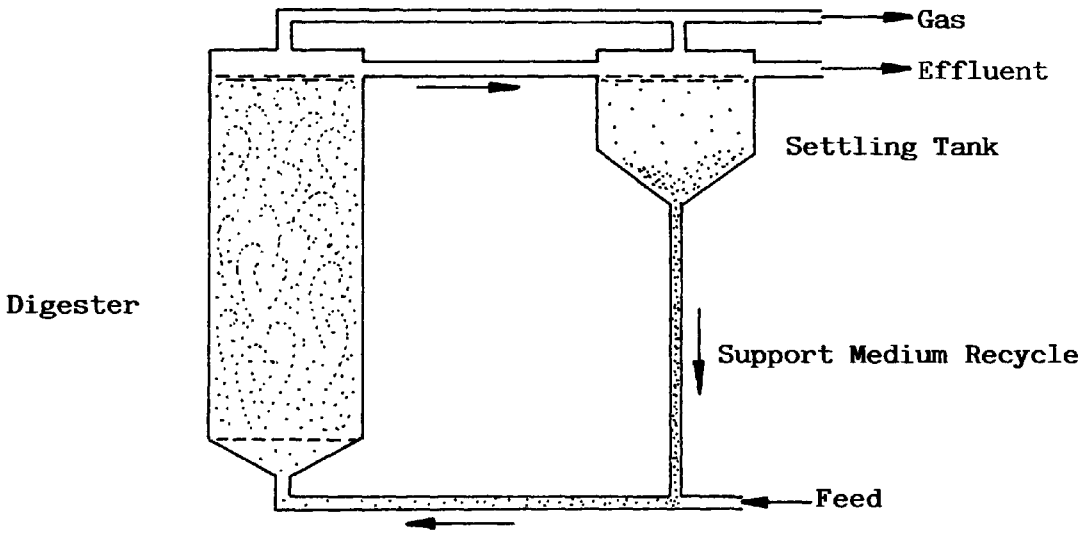


Figure 1.6 Upflow Anaerobic Sludge Blanket

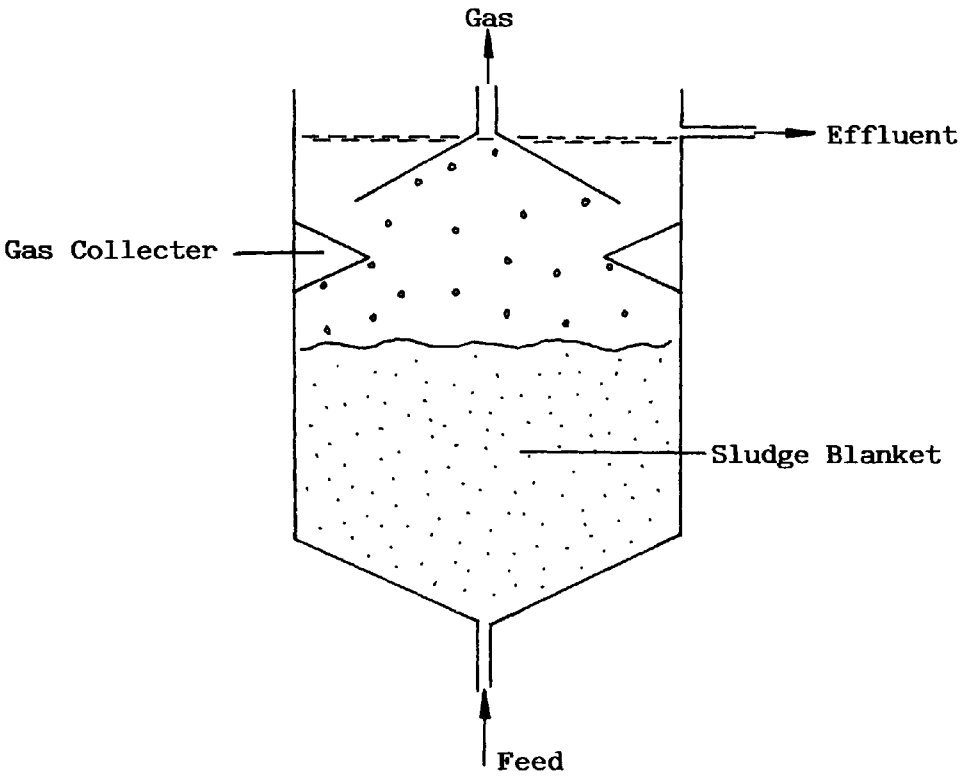
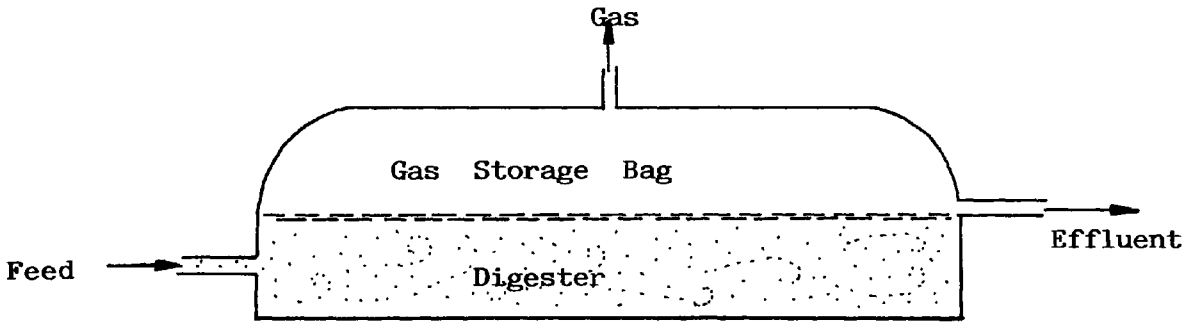


Figure 1.7 Plug Flow Type Digester



CHAPTER 2

CONSTRUCTION, OPERATION AND MONITORING OF PERCOLATING PACKED BED DIGESTERS

2.1 Digester Construction

Eight identical laboratory scale digesters of approximately 10 litre volume were constructed of perspex, and joined with perspex cement, as is shown in Fig.2.1.

The digesters were fitted with tubular sampling ports arranged vertically up the side of the digester and were sealed by means of short lengths of rubber tubing fitted with Hoffman clips. The top and base plates of the digesters were removable, and when in use were sealed with rubber 'O' rings and silicone sealant and firmly bolted. The base of the digester had smooth inclined surfaces to facilitate the flow of liquor from the digester and into the recirculation system. The top plate of the digester had three 5mm diameter ports. The central port being used for the return of recirculated liquor to the digester, a further port was used for the gas to exit and the third as a manometer connection to ensure the digester was gas tight.

Solid substrate within the digester was loaded onto a perforated perspex plate, situated on pegs in the digester wall and was in close contact with the wall. The support plate had 2mm diameter holes at regular intervals, allowing liquor to flow from the bed but retaining large straw fibres. An identical perforated distribution plate was placed above the solid substrate to

facilitate the distribution of recirculated liquor over the bed.

As the base and top plates of the digesters were removable, two or more of the main digester bodies could be bolted vertically together and with the addition of standard top and base plates be operated in the same manner as a conventional single unit (Section 2.3.1) but utilising a proportionately increased weight of solid substrate.

2.1.1 Digester Temperature Control

The digesters were housed inside constant temperature cabinets constructed of 10cm thick polystyrene sheets. The temperature was controlled using electrical fan heaters and heating plates and adjustable thermostats. Internal digester temperature was monitored using NiCr/NiAl thermocouples mounted in a mid-height side port and a Type 1601.1 Electronic thermometer (Comark Electronics Ltd., Littlehampton). By this method fluctuations in digester temperature from the set point were never greater than ±1°C.

2.1.2 Liquor Recirculation

Liquor recirculation was conducted using Watson-Marlow MHRE 100 flow inducers (Falmouth, U.K.). These were situated outside the heating cabinets. Liquor flowed from the digester into rubber tubing, once outside the cabinet this was protected by foam rubber insulation. The tubing was connected via a plastic 'T' piece to 3.2mm internal diameter silicone rubber tubing which passed through

the flow inducer. A second 'T' piece was also connected to rubber tubing, returning the liquor stream to the top of the digester. At each 'T' piece a liquor sampling port was inserted. Liquor recirculation was continuous at the required rate, the silicone tubing being replaced weekly to avoid problems caused by its splitting. Individual pumps were calibrated before use to ascertain the required pump setting corresponding to the required liquor flow rate.

2.2 Digester Contents

Percolating packed bed digesters of the type used in this study, consisted of two phases; the solid substrate (solid phase) and the liquor phase which contained the initial inoculum of bacteria.

2.2.1 Solid Substrate

The solid substrate used was a mixture of dairy cattle manure and wheat straw produced during the overwintering of dairy cattle at New House Farm, Cardiff. This was collected in batches and stored frozen at -20 degrees celsius until required. Due to limited storage space each batch was found to have a slightly different composition, with a total solids content varying between 21.15 and 27.08%. However for each individual study the substrate used was from a single batch. No attempt was made to standarise the feed composition by the addition of water as the relative proportions of constituents also varied. It was felt that the

digesters would operate at feed solids within this range, and the optimum conditions obtained with one batch of feed would be the same as that for a different batch. Because of these variations the feed composition used is given separately in each section of the experimental results.

2.2.2 Inoculum

The inoculum was obtained from a conventional 5 litre stirred tank reactor operating on separated cattle slurry with a total solids content of 3.45% and volatile solids content of 81.01% of the dry matter. The digester was operated at 30°C and a 60 day retention time and fed five times per week (Monday - Friday), producing between 1.7 and 2.3 litres of biogas per day at an average methane content of 64.19% during periods of stable operation. In this way an active inoculum was always obtained. After removal of part of the digester volume for use as an inoculum, increased amounts of feed were added to bring the total digester volume back to 5 litres. Further inoculum was not removed until stable operation had been attained.

Effluent removed from this digester was diluted by the addition of an equal volume of water and was then used to inoculate percolating packed bed digesters. The average inoculum composition is shown in Table 2.1.

2.2.3 Sampling of Digester Contents

The solid manure-straw substrate was sampled before and after digestion, no satisfactory method was found using this particular digester design of sampling contents during the course of digestion. Liquor was taken from the recirculation system at the sample port preceding the flow inducer, so any effect of the liquor passing over the flow inducer rollers would not affect the results. Samples were taken from the recirculation stream when required throughout the course of digestion.

2.3 Digester Operation

2.3.1 Batch Mode Operation

Digesters were loaded with a specified weight of solid material. To this a known volume of liquor, which provided the inoculum was added. The digester was then sealed and the head space flushed with oxygen free nitrogen (B.O.C. Ltd., Port Talbot) to provide anaerobic conditions. The digester was then tested for gas leaks using a manometer and recirculation of the liquor commenced (Fig.2.1). Batch experiments were conducted for periods of up to 70 days.

Table 2.1 Average Inoculum Composition

	Mean Value	Standard Deviation
Total Solids (% wet weight)	1.41	0.23
Volatile Solids (% of T.S.)	71.49	1.58
Volatile fatty acids (ppm)	395.20	61.09
Ammoniacal nitrogen (ppm)	356.47	95.08
Total nitrogen (ppm)	866.29	102.44
pH	7.84	0.26
Alkalinity ($mg.l^{-1}$ $CaCO_3$)	3241.67	524.01
Lipids ($mg.l^{-1}$)	0.39	0.05
Cellulose (% wet weight)	0.47	0.15
Adenosine 5' triphosphate ($\underline{M} \times 10^{-6}$)	3.52	0.47

2.3.2 Semi-continuous Mode Operation

During semi-continuous operation of digesters an uninoculated digester (containing fresh substrate) was linked via its recirculation system to a digester which had been operating previously in a batch mode (Fig.2.2). No inoculum was added, as the system is self-inoculating. Further digesters could then be added in a similar manner after a specified period of time had elapsed. In this study a maximum of three digesters were operated in this manner at any one time. Thus fresh digesters were linked into the system after each $^1/_3$ retention time, refered to as the cycle time. When a fourth digester was added to the system, the first digester was removed having completed the retention time.

2.4 Sample Analysis

A number of analyses were conducted on both the solid and liquid phase and the biogas produced from the digesters. These analyses described the performance of the digesters, and also allowed any problems such as a build up of toxicants to be elucidated.

2.4.1 Total Solids Assay

Total and suspended solids content of the solid and liquor phases was determined by drying samples to a constant weight using a microwave oven (Hitachi, model MR6050) set to 'defrost' for 2 hours. amples of approximately 10g of solid material and 10ml of liquor being dried in preweighed glass beakers. Floyd (1984) showed this method to give results closely comparable with these achieved using the conventional method of oven drying at 105°C to constant weight, but was markedly more rapid.

In an investigation into the reproducibility of this assay using 10 identical samples of the solid material, a standard deviation of 0.49% was obtained when the mean value was 26.31%. The deviation from the mean will also include any inaccuracies brought by errors in obtaining a representative sample.

2.4.2 Volatile Solids Assay

The volatile solids content of dried samples was determined by the incineration of the sample in a 'Eurotherm' electric furnace (Carbolite Ltd., Sheffield) at 500°C for 30 minutes. Samples were placed in pre-weighed porcelain crucibles, and weighed before and after incineration. The solids lost during incineration were designated volatile solids and correspond approximately to organic matter, although some structural water may be lost at 500°C. Volatile solids were expressed as a percentage of the total solids content of the sample according to the following formula :

$$\frac{(\text{Wt. crucible + dried sample}) - (\text{Wt. crucible + ash})}{(\text{Wt. crucible + dried sample}) - (\text{Wt. crucible})} \times 100\%$$

The percentage volatile solids was used as a measure of the biodegradable fraction of the waste, although the figures will include recalcitrant material such as lignin, which is essentially non-biodegradable in an anaerobic environment (Zeikus, 1980a).

An investigation into the reproducibility of this assay showed there to be a standard deviation of 2.22% for six liquor samples having a mean volatile solids content of 79.73%. For the solid phase, six samples with a mean of 80.63% showed a standard deviation of 3.19%.

2.4.3 Volatile Fatty Acid Determination

2.4.3.1 Colourimetric Method

This method does not differentiate between individual fatty acids present, the results being expressed as acetate equivalents. The method is also susceptible to experimental error due to the numerous procedural steps involved, it is therefore of the utmost importance to standardise the procedure to minimise any experimental error. However, the method does allow rapid analysis of numerous samples, and providing care is taken shows good reproducibility, with a standard deviation of 5.38% of the mean value of 2458ppm for 10 identical liquor samples.

Samples were first clarified by filtration, in the case of solid samples this was preceded by the addition of deionised water followed by liquidizing using a blender (Gallenkamp & Co. London) to give a 1:9 w/w dilution. The method used was then essentially that of Montgomery *et al* (1962). To 0.5ml of the sample filtrate, 1.5ml of ethanediol and 0.2ml 50%v/v sulphuric acid were added. The tubes were rapidly mixed and heated for exactly 3 minutes in a boiling water bath and then cooled in ice cold water. 0.5ml of $100gl^{-1}$ hydroxyammonium chloride and 2.0ml of 4.5M NaOH were then added and the tubes again mixed. The samples were then added to 10ml aliquots of $2gl^{-1}$ ferric chloride with 2%v/v sulphuric acid in 25ml volumetric flasks, which were then made up with deionised water and shaken. After 5 minutes the optical density of the sample at 500nm was determined using a Varian Techtron 635 spectrophotometer. A blank sample containing 0.5ml of deionised

water was used as a reference in the spectrophotometer. Acetate equivalents within the sample were then determined using a calibration curve of acetic acid standards, the calibration being linear up to 10000ppm acetate.

2.4.3.2 Chromatographic Method

Chromatographic separation of volatile fatty acids allowed the concentration of individual VFA's (C-2 to C-6) to be determined. Liquor samples were acidified with formic acid (BDH, 'Aristar' grade) to 10% v/v and clarified by centrifugation for one minute in a microfuge (M.S.E. Instruments, Crawley). The supernatent was then removed, and to it added an equal volume of diethyl ether and the sample shaken 20 times before removal of the ether phase for analysis. 1.5µl of the extracted samples were injected onto a 6ft by 4mm glass column of 15% SP1220/1% H_3PO_4 on 100/120 chromasorb W/AW (Supelco Inc., U.S.A.) using an automatic sampler (Varian Assoc. Ltd., Walton on Thames, U.K.) maintained at 145°C with nitrogen as the carrier gas at a flow rate of 30ml.min^{-1}. The injector temperature was 130°C and the flame ionisation detector 155°C. After 20 consecutive injections the machine was calibrated with a known standard of volatile fatty acids and gave a standard error of less than 2% for each fatty acid.

2.4.4 pH Determination

The pH of the liquor was determined using a Russell combination electrode attached to a Phillips PW9409 digital pH meter. On occasions due to equipment failure a Corning 3D hand held pH meter was used. The interval between sampling and pH measurement was minimised and samples were taken in stoppered containers in order to minimise the effects of the loss of dissolved carbon dioxide on pH.

2.4.5 Determination of Alkalinity

As a measure of the buffering capacity of the liquor, total alkalinity was determined by titrating 10ml liquor samples with 1M HCl to pH 4.5 (HMSO, 1972). The results were expressed in terms of mgL^{-1} dissolved calcium carbonate using the following formula:

$$\frac{50000 \times \underline{M}\ HCl \times \text{volume HCl(ml)}}{\text{Sample volume (ml)}} = mgl^{-1}\ CaCO_3$$

2.4.6 Determination of Ammoniacal Nitrogen

Ammoniacal nitrogen was determined by distillation (HMSO, 1972). As in the case of volatile fatty acid determination, solid samples were diluted 1:9 w/w in deionised water and homogenised. Samples were first diluted with an equal volume of 0.1M HCl and then centrifuged at 4000 rpm for 20 minutes in a bench centrifuge (M.S.E. Instruments, Crawley). A 1ml sample of the supernatant was then transfered to a Markham Steam Distillation unit and 3.5ml of

10M NaOH added. 20ml of distillate was then collected into a glass beaker containing 5ml boric acid indicator consisting of 20g boric acid, 200ml ethanol, 10ml mixed acid indicator made up to a total volume of 1 litre with deionised water. The distillate was then titrated against 0.01M HCl, a blank titre being used to determine any background reading. The concentration of ammoniacal nitrogen was then determined according to the following:

$$\frac{13999.5 \times \text{dilution factor} \times \text{M HCl} \times \text{volume HCl}}{\text{sample volume}} = \text{ppm ammoniacal nitrogen}$$

Replicate samples (10) showed a standard deviation of 6.02% (mean = 1734.43 ppm) for the solid phase and 5.57%(mean = 459.98) for the liquor phase.

2.4.7 Determination of Total Nitrogen

A 1ml sample of the liquor phase, or a known weight of the solid phase (normally between 0.1 and 0.3g) was first placed in a Kjeldahl digestion tube and 2.0ml of the digestion mixture ($10g.L^{-1}$ Selenium dioxide in 50% v/v Sulphuric acid) added. The flask was then gently heated using an adjustable gas flame until all frothing had ceased and then more strongly until a clear solution was obtained (HMSO, 1981). The flask was then cooled and its contents quantitatively transfered with the aid of deionised water washes into a Markham Still. 10ml of 10M NaOH was then added and distillation, collection and titration conducted as described in section 2.4.6, and the results in ppm of total nitrogen obtained as previously described.

An investigation into the reproducibility of this assay

showed there to be a standard deviation of 7.46% of a mean value of 955.96 ppm for the liquor phase and 7.72% (mean = 5329.47ppm) for the solid phase, six replicate samples being used in each case.

This method for the determination of Total nitrogen is based on the conversion of nitrogenous material present in the sample to ammonia (with retention of any ammoniacal nitrogen). Thus if the ammoniacal nitrogen concentration is subtracted from that of total nitrogen, the difference (non-ammoniacal nitrogen) can be used to estimate crude protein content after multiplication by a factor of 6.25 (Allen et al, 1974)

2.4.8 Holocellulose Assay

Holocellulose was determined by the method of Allen et al (1974). This is a delignification procedure, preserving both hemi- and alpha-cellulose, providing an estimate of the total polysaccaride fraction. A known weight of sample (usually between 1 and 2g) was placed in a 250ml heating tube and approximately 20ml of deionised water added. This was then heated in a Techne DB4 Dry Block heating unit (Techne Ltd., Cambridge) at 75°C for 4 hours with 0.3g sodium chloride and 1ml 10% v/v acetic acid being added initially and after 1, 2 and 3 hours. After 4 hours the samples were removed and cooled.

When cool the samples were centrifuged for 25 minutes at full speed in a bench centrifuge, the supernatant discarded and the pellet resuspended in deionised water. This was then repeated twice with deionised water, twice with acetone and once with diethyl ether. The final pellets were washed into preweighed

beakers with diethyl ether to ensure complete transfer, and the ether evaporated overnight, and then further dried in a dessicator and their weights determined. The volatile solids content of the extract was then determined and the weight of non-volatile matter subtracted from the results, and the percentage wet weight of holocellulosae obtained.

This assay was found to be highly reproducible, showing a standard deviation of 3.87% of the mean value (10 samples of total solids content of 25.75%) of 13.33%.

2.4.9 Determination of Adenosine 5' triphosphate

Adenosine 5' triphosphate (ATP) levels within the solid and liquid phases were determined using a bioluminescence assay. Liquid and solid samples were taken under a constant flow of oxygen free nitrogen (BOC, Port Talbot) to maintain anaerobic conditions. The ATP extraction procedure used was after that of Wallace and West (1982).

An equal volume (or weight) of ice-cold 0.5M H_2SO_4 was first added to the sample, and the mixture rapidly homogenised and then held on ice for 2 minutes. The solution was then decanted into 1.75ml Eppendorf vials and centrifuged at full speed in a Microfuge for 10 minutes at 4°C. 0.4ml of the supernatant was removed and neutralised with 0.2ml 1M NaOH, 0.4ml 1M Tris-HCl (pH7.8) and 1.0ml deionised water. The step was conducted immediately after centrifugation to minimise ATP breakdown (Forsberg & Lam, 1977). The extract was then diluted (x 10) in buffer (100mM Tris-acetate pH7.8 with 2mM EDTA). This buffer was also used in determining

background bioluminescence. A 0.2ml sample was then taken and 0.04ml of ATP monitoring reagent (L.K.B. Instruments, Croyden) added and the bioluminescence determined using a Pico-lite luminome ter (Hewlett-Packard, Wokingham) which was maintained at 20°C using a flow heater and cooler (Grant Instruments, Cambridge) to ensure reproducibility. The result was then converted into ATP concentration using a linear calibration curve for ATP concentrations between 10^{-10} and 10^{-6}M (Fig.2.3) which was verified using an ATP standard on each occasion. The ATP concentration obtained cannot be directly related to bacterial numbers due to differential bacterial activity and ATP pool sizes. For example Robertson and Wolfe (1970) found the pool size to vary between 0.9 and 2.0µg ATP/mg viable dry cell weight in continuous culture, and Lundin and Thore (1975) obtained 0.8 - 3.5µg ATP/mg viable dry cell weight for acid forming bacteria. However, where samples are taken from digesters having operated for the same time period, any major differences in ATP concentration or trends in ATP concentration can be considered to reflect variation in bacterial numbers.

2.4.10 Total Lipids Assay

The assay used was based on that of Bligh and Dyer (1959). 8ml of wet sample (or 8ml of diluted and homogenised solid sample) was homogenised for 30 seconds with 20ml methanol and 10ml chloroform. A further 10ml of chloroform and 10ml deionised water was then added and homogenised for a further 30 seconds. The chloroform used contained $5mg.l^{-1}$ But yrated hydroxyquinoline as an antioxidant.

The samples were then centrifuged for approximately 10 minutes at full speed in a bench centrifuge to aid separation of the lower chloroform layer and the upper aqueous layer. Solid impurities gathered at the phase boundary and were easily discarded when the samples were transfered to a separating funnel. The chloroform layer was then drawn off and its volume measured, a known proportion of which was then dried overnight in a preweighed watch glass, and the lipids content of the sample determined.

Reproducibility of this assay was found to be poor, and for 10 solid samples with a mean lipids content of $10.5g.Kg^{-1}$ a standard deviation of over 20% was obtained. Analysis of the liquor showed improved reproducibility with a standard deviation of 9.61% being obtained when the mean lipids concentration was $0.33g.l^{-1}$. Due to its poor reproducibility and the very low lipids concentrations within the liquor phase, this assay was only conducted during the initial feasibility study.

2.4.11 Sample Preparation for Scanning Electron Microscopy

Samples to be viewed with the scanning electron microscope (SEM) were prepared by dehydration of the sample followed by critical point drying. 1ml samples of the liquor phase were first diluted five times and filtered through a 0.45μm millipore sieve. The filtered liquor samples and solid samples were then fixed for 30 minutes in 3% glutaraldehyde and then sequentially immersed for 15 minutes in 30%, 50%, 80%, 90%, 95% and 100% ethanol followed by a further 15 minutes in 1:1 ethanol:amyl acetate and absolute amyl acetate. Transfers between solutions were carried out rapidly to

avoid drying of the sample. Samples in amyl acetate were then transfered into a critical point drying pressure vessel (Polaron Ltd, Watford). The amyl acetate was displaced by flushing with liquid carbon dioxide, in which the sample was immersed for 1 hour. Carbon dioxide was then taken past its critical point of 31.5°C and 1100 psi by heating with warm water. In this way carbon dioxide changes instantly from liquid to gasous phase, with no liquid meniscus passing through the cells, and thus avoiding cellular damage. Dried samples were then sputter coated with gold before viewing in a Scanning electron microscope (Cambridge Instruments, model no. 150).

Two further methods of sample preparation for scanning electron microscopy were also examined in an attempt to simplify the above procedure. The first involved air drying after fixing in glutaraldehyde, and the second followed the alcohol dehydration procedure shown above, followed by air drying (Robinson et al, 1984). The results obtained are shown in plates 2.1, 2.2, and 2.3 and clearly show the requirement for critical point drying to avoid cellular damage which occurs on dehydration of the sample when the two simplified methods were used.

2.4.12 Proportional Gas Production from the solid and Liquid phases

A 1 litre sample of liquor was drawn from the digester after the specified operation time into a conical flask under a constant flow of oxygen-free nitrogen. The flask was then sealed using a rubber bung with two access ports, of which one was used to sample the head space gas for its methane content and the second

connecting the flask via rubber tubing to an inverted 50ml graduated cylinder filled with 0.1M HCl to reduce carbon dioxide dissolution. The flask was placed in an incubating oven at 30°C and gas production monitored for 2 hours. By the use of control digesters it was possible to evaluate the gas production from a whole digester over this time period. The volume of gas produced from the liquor sample was then multiplied to correspond to the total liquor volume of the digester. This value was then subtracted from the gas produced by a control digester and the difference assumed to have originated from within the solid phase.

Measurement of the gas composition allowed any variation in the methane content of the biogas from the solid and liquid phases to be elucidated, which could indicate any variation in the bacterial populations of the two phases.

2.4.13 Assessment of Bacterial Levels of the Liquor Phase

A study was conducted to assess the levels of methanogenic and non-methanogenic (hydrolytic and acidogenic) bacteria within the liquor phase. This was conducted using a modified Hungate technique (Bryant, 1972) where anaerobic conditions are maintained by displacing all air in culture tubes with an appropriate gas. The gas is passed into the vessel by means of syringe points (or Pasteur pipettes) attached by rubber tubing to the gas source. Entrance of oxygen is prevented by closing the vessel with a rubber stopper as the points are removed. A reducing agent ensures low oxidation-reduction potential and resazurin is added as an indicator of anaerobiosis. The general procedure used was

identical for both methanogen and non-methanogen cultivation, liquid media being used in both cases.

2.4.13.1 Cultivation of Methanogenic Bacteria

The media used was after that of Balch and Wolfe (1976) (Table 2.2). The media was first sterilised by autoclaving at 121°C for 20 minutes, and the reducing agents (cysteine hydrochloride and sodium sulphide) sterilised separately and then aseptically added. The flask was then placed on a hot plate/stirrer (the seal having been slightly dislodged) and three syringe points were inserted into it, two below the surface of the media and one above and were connected to a supply of commerical 'oxygen free' nitrogen (B.O.C. Ltd, Port Talbot). This was found to be sufficient to maintain anaerobic conditions in the media. Two further syringe points were directed into a sterile 12ml glass culture tube into which a 9ml aliquot of the media was then pipetted from the flask to the culture tube which was immediately closed with a rubber stopper and placed in a glove box through which a constant flow of 'oxygen free' nitrogen was passed. This procedure was then repeated to give the required number of tubes, in this case 22, to allow serial dilutions between 10^{-1} and 10^{-11} and two controls.

Two 1ml samples of the liquor were then obtained by inserting 1ml sterile syringes through the rubber tubing of the liquor recirculation system. The liquor was injected through the rubber septa of the culture tubes, and thoroughly mixed. A 1ml sample was then removed using a fresh syringe and injected into the

Table 2.2 Media Composition for the Culture of Methanogenic Bacteria

0.45 $g.l^{-1}$	KH_2PO_4
0.45 $g.l^{-1}$	K_2HPO_4
0.45 $g.l^{-1}$	$(NH_4)_2SO_4$
0.90 $g.l^{-1}$	NaCl
0.18 $g.l^{-1}$	$MgSO_4$
0.012$g.l^{-1}$	$CaCl_2$
0.002$g.l^{-1}$	$FeSO_4$
2.0 $g.l^{-1}$	Sodium formate
2.5 $g.l^{-1}$	$NaHCO_3$
2.0 $g.l^{-1}$	Yeast extract
2.5 $g.l^{-1}$	Sodium acetate
0.5 $ml.l^{-1}$	Iso-butyric acid
0.5 $ml.l^{-1}$	Methyl-butyric acid
0.5 $ml.l^{-1}$	Iso-valeric acid
0.5 $ml.l^{-1}$	Valeric acid
0.001$g.l^{-1}$	resazurin
20 $ml.l^{-1}$	vitamin solution (see Table 2.4)

Added separately in 10ml deionised water :

0.5 g	L-cysteine-HCl
0.5 g	Sodium sulphate

next culture tube, and repeated to obtain the desired serial dilution. The control tubes were set up by inserting and removing 1ml of media from each to determine if any contamination had occured. The tubes were then removed from the glove box and placed in a sealed container flushed with 'oxygen free' nitrogen and incubated at 30°C. After 6 days the tubes were removed and the headspace gas analysed for methane and carbon dioxide, the presence of the former indicating methanogens to be present.

Any traces of oxygen which may have been present in the media or sample will be removed by facultative anaerobes present. However, the methanogens present may have been affected leading to an underestimation of their numbers.

2.4.13.2 Cultivation of Non-Methanogenic bacteria

A yeast extract media after that of Braun *et al* (1979) was used (Table 2.3). Duplicate serial dilutions were conducted as previously described. The culture tubes were incubated at 30°C for 24 hrs, as it was believed that this period would not be sufficient for any significant growth of methanogens to occur. The optical density (at 600nm) of the cultures was then determined using media blanks as references, the presence of bacteria being indicated at the lowest dilution where a positive reading was obtained. The results may indeed be an underestimate due to the presence of attached bacteria.

Table 2.3 Media Composition for the Cultivation of Non-Methanogenic Bacteria

2.0 $g.l^{-1}$	Yeast extract
20 $ml.l^{-1}$	Vitamin solution (see table 2.4)
20 $ml.l^{-1}$	Mineral solution (see table 2.4)
10.0 $g.l^{-1}$	$NaHCO_3$
1.0 $g.l^{-1}$	$MgSO_4$
1.0 $g.l^{-1}$	NH_4Cl
5 $ml.l^{-1}$	Potassium phosphate buffer
1 $ml.l^{-1}$	0.1% resazurin

Added separately in 10ml deionised water

0.5 g	L-cysteine-HCl
0.5 g	Sodium sulphate

Table 2.4 Vitamin and Mineral Solutions used as Media Additives

Mineral Solution (Bryant and Burkey, 1953)

0.3% w/v	K_2HPO_4
0.3% w/v	KH_2PO_4
0.6% w/v	$(NH_4)_2SO_4$
0.6% w/v	NaCl
0.06% w/v	$MgSO_4$
0.06% w/v	$CaCl_2$

Vitamin Solution (after Van den Berg, 1977)

2 $mg.l^{-1}$	Biotin
2 $mg.l^{-1}$	folic acid
10 $mg.l^{-1}$	pyridoxine HCl
5 $mg.l^{-1}$	riboflavin
5 $mg.l^{-1}$	thiamine
5 $mg.l^{-1}$	nicotinic acid
5 $mg.l^{-1}$	pantothenic acid
0.1 $mg.l^{-1}$	vitamin B_{12}
5 $mg.l^{-1}$	p-aminobenzoate
5 $mg.l^{-1}$	thiocitic acid

2.4.14 Analysis of Particle Size Distribution within the Liquor Phase

Liquor particle size distribution was estimated by passing 1 litre of liquor through a nest of three sieves of mesh sizes 1000μm, 250μm and 75μm, the remainder passing through the smallest mesh was also collected. The dry weight of material retained on each sieve was determined by drying for 2 hours in a microwave oven set to defrost. The total solids content of an identical liquor sample was also determined (Section 2.4.1). Thus the percentage dry weight of particles greater than 1000μm, 250-1000μm, 75-250μm and those less than 75μm could be estimated.

2.4.15 Carbon : Nitrogen Ratio

The carbon : nitrogen ratio has been shown (eg. Hills, 1979) to be of great importance to the digestibility of wastes during anaerobic digestion. As lignin was not estimated during this study all results quoted are the non-lignin carbon : nitrogen ratio. The weights of cellulose, protein, volatile fatty acids and lipids were first determined for the solid waste used and their carbon contents (in grams) estimated assuming lipids and VFA's to contain approximately 70% carbon, cellulose to contain 44% carbon and protein to contain 56% carbon. These values were then summated. The weight of nitrogen added can then be estimated from the substrate's total nitrogen content. Thus by dividing the weight of carbon added by that of nitrogen added the non-lignin carbon : nitrogen ratio is obtained.

2.4.16 Gas Volume

The volume of gas produced was measured daily using a Wet type gas meter (A. Wright and Co., Tooting) model DM3A filled to the calibration mark with Castrol insulating oil. Gas samples were taken from a sample port in the rubber tubing connecting digester to gas meter.

2.4.17 Gas Composition by Gas Chromatography

The methane and carbon dioxide content of the digester gas was monitored daily using a Varian 6500 Gas Chromatograph (Varian Associates Ltd., Walton on Thames). This utilised a 2 metre by 3mm diameter stainless steel column containing 80-100 mesh Porapak T, a thermal conductivity detector and Helium carrier gas at a flow rate of $30ml.min^{-1}$ at 60°C. The gas was sampled using a 20ml plastic syringe with a 3-way tap, and immediately injected into the 0.25ml injection loop of the chromatograph.

2.4.18 Biogas Hydrogen Sulphide Concentration

Hydrogen sulphide levels in the biogas were monitored using type 5b Draeger Tubes (Draegerwerk, FRG) which function due to a colour change, the magnitude of which is dependent on the hydrogen sulphide concentration in the biogas.

Figure 2.1 Digester Operation Batch Mode

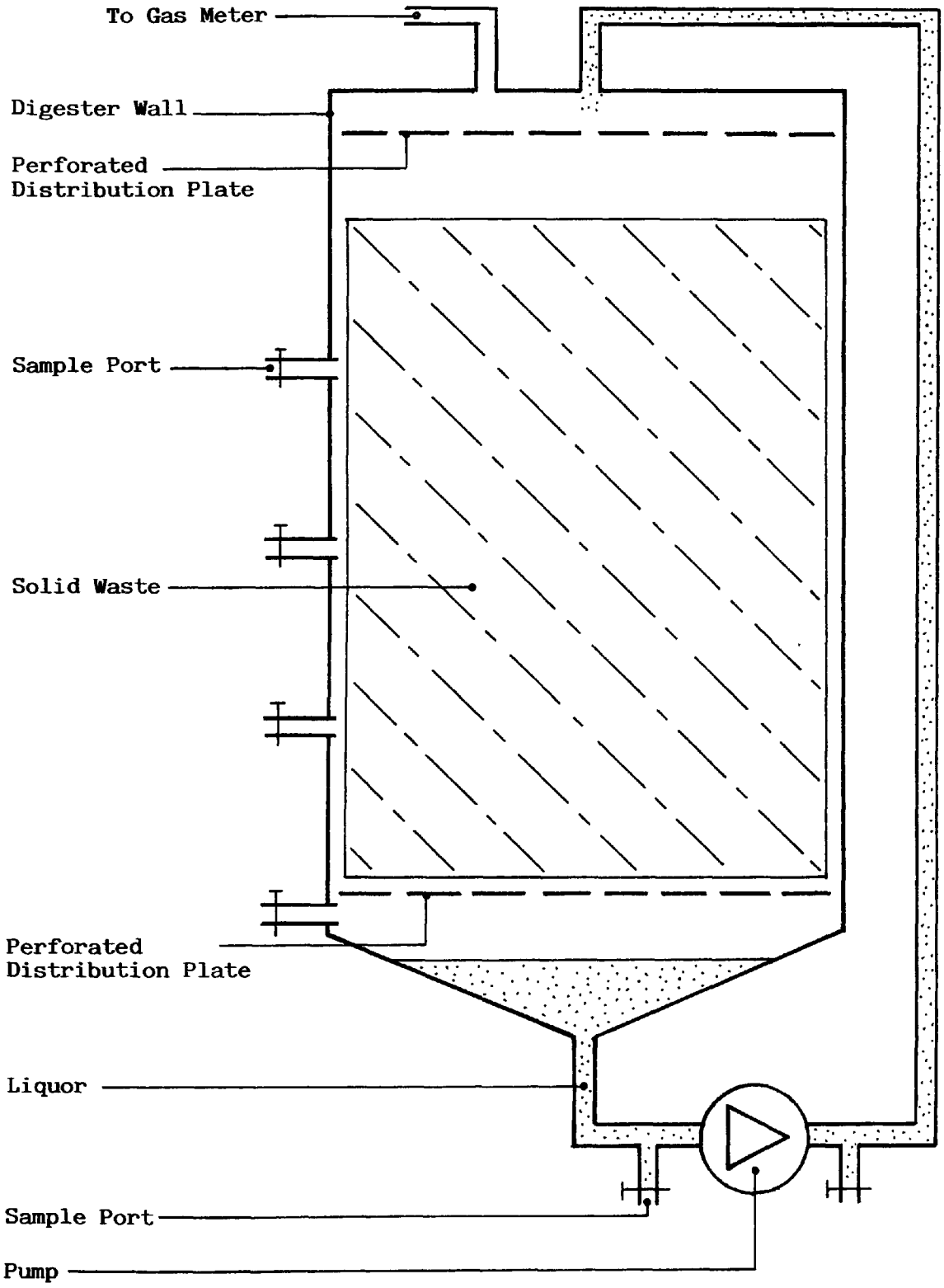


Figure 2.2 Digester Operation Semi Continuous Mode

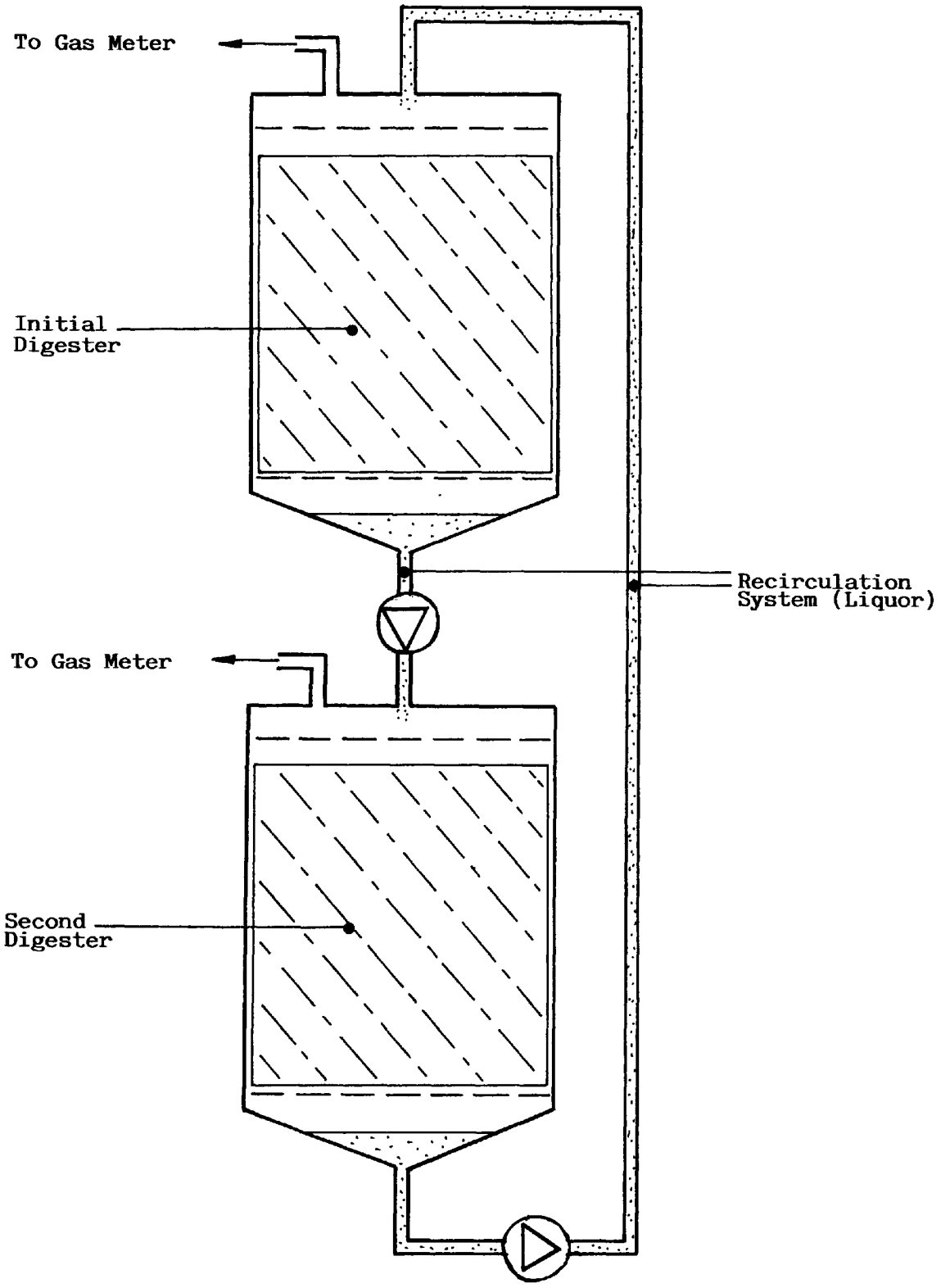


Figure 2.3 Calibration Curve of Adenosine-triphosphate Standards

(Bar indicates range of results)

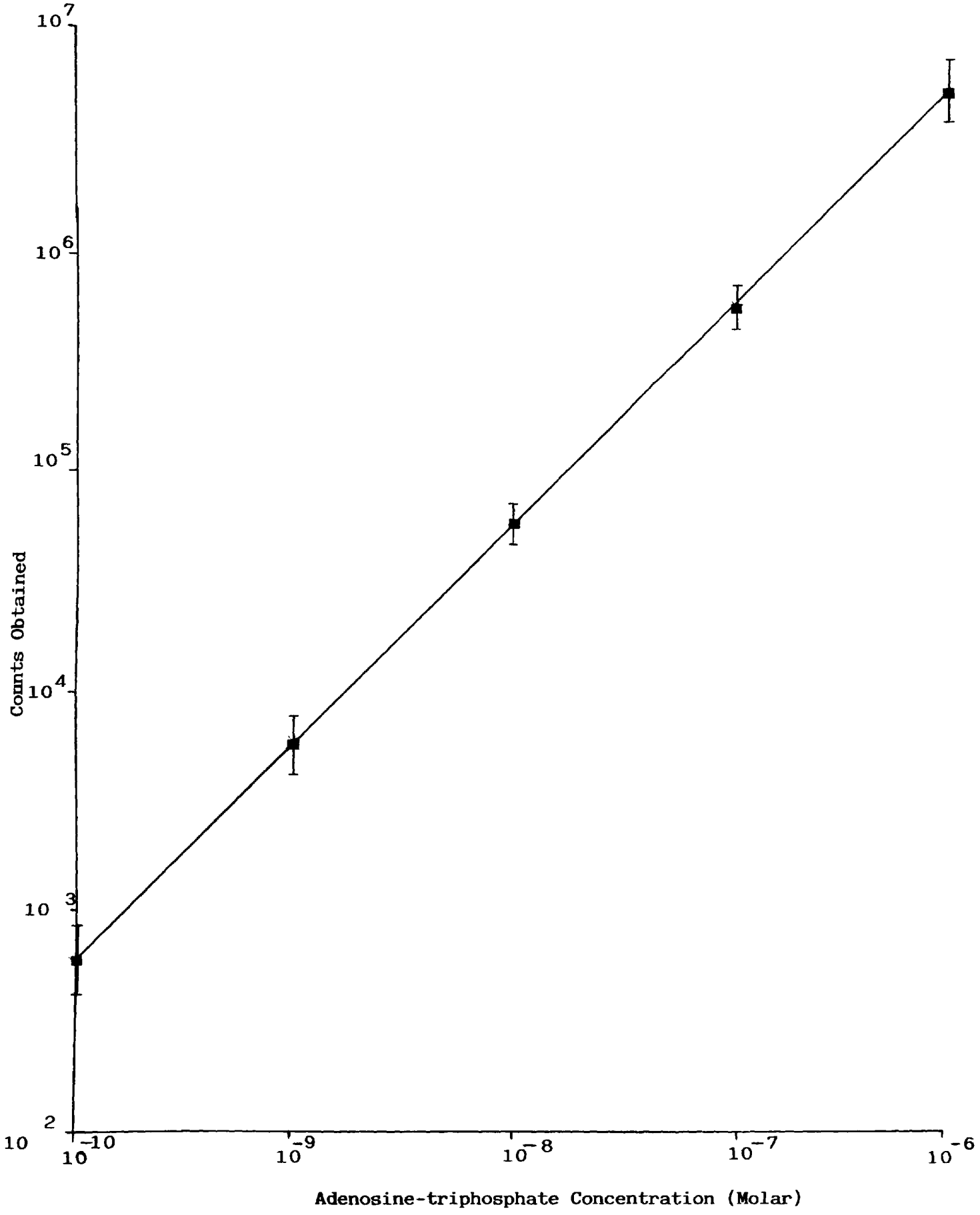


Plate 2.1 Scanning Electron Micrograph of a Liquor Sample : Prepared by Dehydration and Critical Point Drying

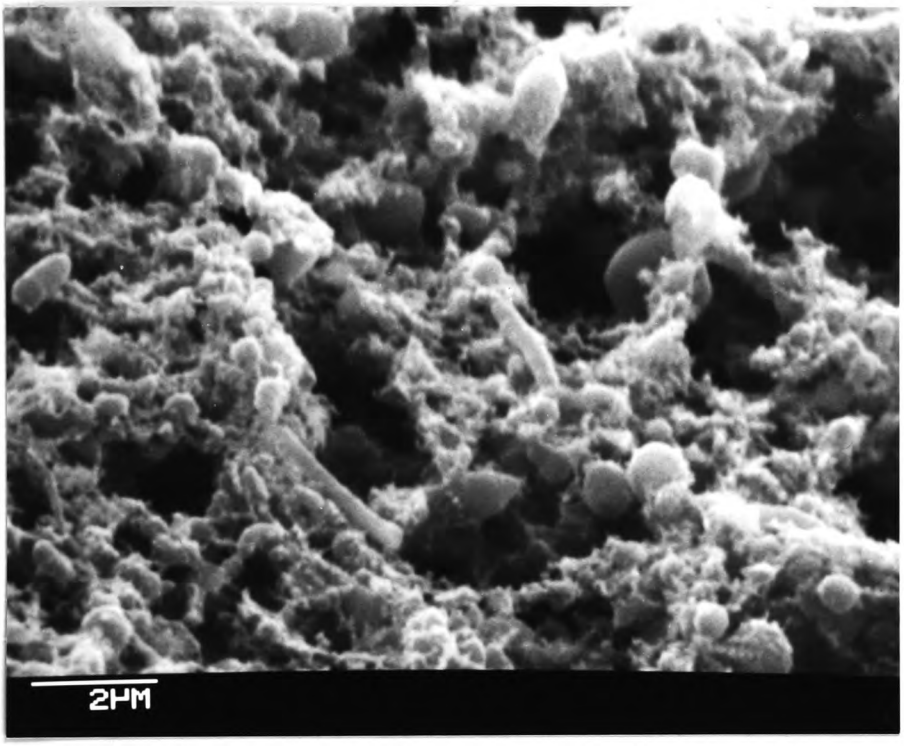


Plate 2.2 Scanning Electron Micrograph of a Liquor Sample : Prepared by Air Drying

A: Methanosarcina like aggregate of bacteria

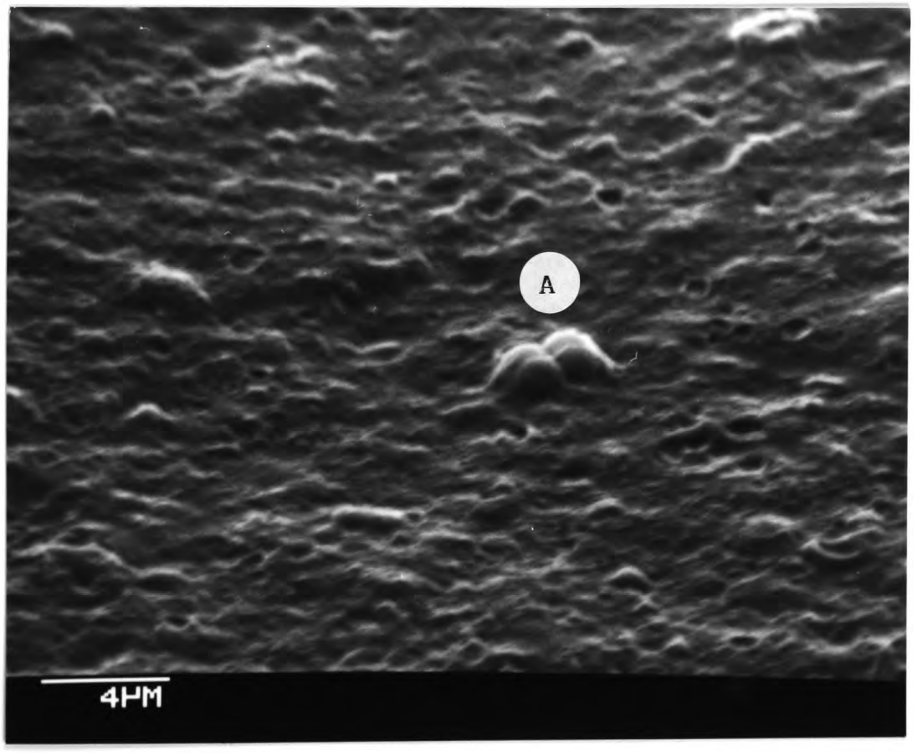
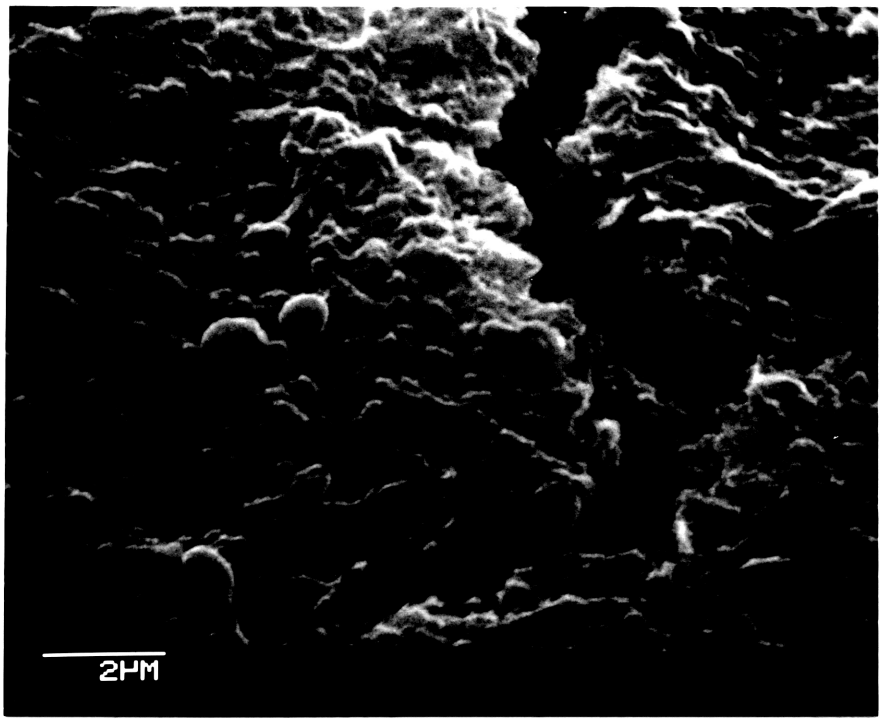


Plate 2.3 Scanning Electron Micrograph of a Liquor Sample : Prepared by Dehydration and Air Drying (Robinson et al, 1984)



CHAPTER 3

FEASIBILITY STUDY - BATCH PROCESS

3.1 Introduction

The main objective of this study was to investigate the operability and performance of percolating packed bed digesters using high solids dairy manure.

A precept of operating anaerobic digesters is that the presence of volatile fatty acids and/or high ammoniacal nitrogen could cause souring particularly at start up.

Virtually no previous work has been carried out on units of this design, therefore a preliminary experimental programme was necessary in order to verify operability and also to gain practical experience of analytical techniques used in monitoring digester performance. It was also necessary to determine approximate rates of gas production, changes in liquor composition and the rate of solids degradation in order to determine the time scale over which experiments should be conducted and the frequency of analysis required for both the liquid and solid phases.

3.2 Digester Operating Conditions

These experiments utilised 3kg of solid substrate to which was added 3 litres of liquor (obtained as described in sections 2.2.1 and 2.2.2) which provided the inoculum. The liquor was recirculated at a rate of 3 litres/hr and the temperature

maintained at 30°C.

At this stage each experiment was conducted on three occasions over 40 day periods followed by two further experiments having a duration of 70 days.

3.3 Digester Monitoring

Gas production and composition, together with the internal digester temperature was monitored daily. The solid phase was analysed prior to and after digestion, and the initial composition is shown in Table 3.1.

Table 3.1 Initial Composition of Solid Substrate

	mean value	standard deviation
Total solids (% wet wt.)	26.33	0.63
Volatile solids (% of T.S.)	80.07	1.31
Volatile fatty acids (ppm)	2643.75	707.56
Ammoniacal nitrogen (ppm)	1676.58	103.98
Total nitrogen (ppm)	6884.66	357.71
Cellulose (% wet wt.)	14.18	0.68
Lipids (g/kg)	9.32	1.81
non-lignin Carbon : Nitrogen	17:1	

Constituents of the liquor phase were monitored as follows:

Constituent	Times Sampled per Week
Volatile Fatty Acids	3
Ammoniacal Nitrogen	3
Total Nitrogen	3
pH	3
Total Solids	2
Volatile Solids	2
Alkalinity	1

Liquor cellulose concentration was determined before and after digestion.

3.4 Results and Discussion

3.4.1 Operational Assessment

During the preliminary investigation which involved a total running time in excess of 250 days, no souring or toxicity problems leading to digester failure occurred. At no time did any part of the system (packed bed or recirculation stream) become blocked. It was observed that even after 70 days of operation the straw fibres present in the bed retained enough rigidity to prevent compaction. Indeed it was found that the bed and perforated support plate acted as a mechanical filter for the liquor (Chapter 5). The inoculum had an average initial cellulose concentration of 0.635% wet weight, after 40 days of operation the average concentration was 0.032% corresponding to a 95% loss in liquor cellulose content. The inoculum contained straw fragments between 1 and 2mm in length,

which were absent from the liquor immediately after recirculation had commenced.

Due to the manner of construction of the digesters, being constructed of rolled perspex sheets glued down a vertical seam, it was found that after a few weeks operation, minor temperature fluctuations within the insulated cabinet resulted in this seam splitting and the loss of anaerobic conditions.

The problem was overcome by using flexible silicone jointing strengthened with 15cm 'Jubilee' clips.

It was noted during this development stage that the loss of anaerobic conditions was not as detrimental as other workers have suggested. Scott et al (1983) have reported that methanogenesis in anaerobic digesters was irreversibly inhibited by O_2 concentration of less than 30nM. In the system studied here however, methane production commenced within 24 hours of sealing the split digester and had reached its original composition of around 60% methane after only four days. This may reflect a higher level of facultative anaerobic bacteria present, or that the methanogenic bacteria present have a lower oxygen sensitivity, or being attached to the solid bed are afforded some form of protection from the effects of oxygen.

3.4.2 Gas Production and Yield

Gas production commenced immediately upon achieving anaerobic conditions (Fig.3.1). Daily gas production was low for the first three days (averaging 1.7 litres/day). From days 3 to 12 however, daily gas production was maintained at a high level of

between 3.5 and 4.25 litres/day. Gas production then declined rapidly with approximately 1 litre/day being produced after 40 days. This trend in gas production is similar to that found by Wong-Chong (1975) using dairy manure of 20.8% T.S. in a batch digestion. A rapid almost linear increase in gas production was noted, followed by a steady high rate of production, and finally an almost exponential decline. The gas yields obtained are shown in Table 3.2.

Table 3.2 Gas Yields obatined during Initial Feasibility Study

Average Weight V.S. added (kg)	0.632
Average Weight V.S. destroyed after 40 days (kg)	0.163
Average Weight V.S. destroyed after 70 days (kg)	0.198
Average Cumulative Gas Production after 40 days (litres)	104.9
Average Cumulative Gas Production after 70 days (litres)	132.8
Average Gas Yield (m^3/kg V.S. added) after 40 days	0.166
Average Gas Yield (m^3/kg V.S. added) after 70 days	0.210
Average Gas Yield (m^3/kg.V.S. destroyed) after 40 days	0.645
Average Gas Yield (m^3/kg V.S. destroyed) after 70 days	0.671

The slight variation in gas yield per kg volatile solids destroyed after 40 and 70 days is possibly due to differential rates of digestion for the constituents of the substrate, with different components being destroyed after for example 40 days than before this time.

If the gas yields (per kg volatile solids added) are

considered over 10 day periods (Fig.3.2). The proportional gas yield decreased with increasing rapidity throughout the course of digestion. From these preliminary results it was decided to terminate all future batch experiments after 30-40 days of operation, due to the low rates of gas production obtained thereafter, and that this period was sufficient to obtain the information pertaining to each set of experimental conditions. Studies show that the total gasification of the biodegradable material could take between 100 and 300 days (Jewell (1980)), and Hills (1980a), using high solids dairy manure found that 85% of biogas was produced within the first 45 days of digestion.

The results obtained are comparable with those of other workers. For example Kottowitz and Schulte (1982) obtained a yield of 0.3 m^3/kg VS added using beef manure at a retention time of 53 days. It should be noted however that direct comparison with results pertaining to wastes other than dairy manure are not strictly valid, as it has been shown (Hill, 1982) that dairy manure has a biodegradability of only 50% that of beef manure and 40% that of poultry and swine wastes.

3.4.3 Gas Composition

Methane content of the gas reached its maximum level after between 5 and 7 days of operation (Fig.3.3). The methane concentration achieved and maintained throughout each run was on average 62.6% and the standard deviation was found to be 2.7% when all experimental results were analysed. The rapid onset of methane production (it accounts for around 30% of the biogas on only the

second day of operation) is an indication of the well adapted inoculum used. Hills (1980a) found it took 10 days for the flora of municipal waste digesters to acclimatise to cattle waste. This could be a significant loss of gas production in a full scale process, and adversely affect its economic viability.

3.4.4 Solids Losses

Analysis of the solid phase after digestion showed the following solids losses (Table 3.3). These results clearly confirm that there is a little advantage in operation for periods longer than 40 days. The solids losses were substantial and are comparable with the findings of other workers using high solids waste, Hills (1984) obtaining volatile solids reductions of 34.6% during a 100 day batch digestion of dairy manure, and Wong-Chong (1975) obtaining a 36.3% volatile solids reduction during a 140 day batch digestion. The loss of cellulose was particularly high, being comparable with the results of O'Kelly *et al* (1983) who obtained cellulose reductions of 62% during the two-phase anaerobic digestion of wheat straw, and those of Colleran *et al*, 1980. The high levels of cellulose reduction obtained in the percolating packed bed digester again indicate the importance of using a well adapted seed. Hobson *et al* (1981) reported that straw was not degraded in a conventional digester below a retention time of 20 days due to inappropriately acclimatised bacterial population.

In addition the substrate volume was reduced by approximately 30% during the course of digestion.

Table 3.3 Solids Losses in Initial Feasibility Study

(All results are averages with their standard deviations)

Parameter	Initial Weight (kg)	Weight after 40 days (kg)	Weight after 70 days (kg)	Percentage destruction after 40 days	Percentage destruction after 70 days
Total Solids	0.798 ± 0.021	0.631 ± 0.015	0.607 ± 0.036	20.2%	23.1%
Volatile Solids	0.632 ± 0.027	0.470 ± 0.029	0.435 ± 0.031	25.7%	31.3%
Cellulose	0.425 ± 0.015	0.291 ± 0.010	0.182 ± 0.011	31.5%	57.2%

3.4.5 Changes in Liquor Volatile Fatty Acid Concentration

Liquor volatile fatty acid concentration was monitored three times weekly during these experiments and the results presented are averages from all experiments conducted and are condensed from in excess of 150 individual readings.

Liquor volatile fatty acid concentration (Fig.3.4) shows an initial rapid rise, reaching a maximum of approximately 3800ppm after three days. This was followed by an equally rapid decline to around 2500 ppm on day 5. The high initial level of VFA concentration is reflected in the low levels of biogas production over the first three days of operation (section 3.2). This is most probably due to the inhibition of methanogenic bacteria by the high levels of VFA's and in particular longer chain fatty acids such as propionic acid which could not be differentiated from acetic acid by the colourimetric assay used (Hanaki et al, 1981). After 12 days the VFA concentration had reached a steady level of approximately 1500ppm.

Volatile fatty acids within the solid phase show little change over the course of digestion, averaging 1847 ppm at the end of the digestion period. This concentration is some 300ppm greater than the liquor VFA concentration. As hydrolysis and acid production occur within the solid phase, the acids are treated by attached bacteria on the bed, a proportion of the VFA's however will be carried through the bed and into the recirculation system. Part of this VFA carry over will be treated within the liquor phase, the remainder being recirculated to the bed to be degraded.

3.4.6 Variation in Liquor pH

As was expected during the start-up of percolating packed bed digesters, there was an initial decrease in liquor pH (Fig.3.5) to approximately 7.0 during the first five days of operation, caused by the accumulation of volatile fatty acids. pH then rapidly increased, attaining a steady level of approximately 7.5 after twelve days of operation. This level was then maintained, with random minor fluctuations throughout the course of each experiment. It was initially anticipated that one of the major problems which might be encountered with this type of system where high organic loads are used, was the build-up of volatile fatty acids leading to a drop in pH and souring (inhibition of methanogenesis) of the digester (Rijkens 1981). This occurs due to methanogens having lower specific growth rates than the acid producing bacteria, and being inhibited by the low pH and high redox potential caused by high concentration of H_2 and thus of VFA's (Hobson & Shaw, 1973).

Souring of the digesters did not however occur, and the initial pH decrease and VFA increase was transient, leading only to a minimal loss of biogas production during the first 3 days of operation. This is possibly due once again to the use of acclimatised seed, and also the inherently high buffering capacity of the system. Alkalinity was in all cases consistently in excess of 6000 mg/l of calcium carbonate. Thus the increase in VFA concentration does not induce a drastic change in pH or redox potential, and therefore only a minimal effect on methane production was noted and equilibrium (observed as stable pH and

liquor volatile fatty acid concentration) was rapidly developed.

3.4.7 Effect of Digestion on Nitrogen Concentrations in the Solid and Liquid Phases

The liquor concentration of both ammoniacal and total nitrogen (Fig.3.6) shows a general trend of increasing concentration, with relatively large fluctuations in concentrations occurring around the overall increase.

Over the course of 70 day batch digestions ammoniacal nitrogen increased by approximately 300ppm and total nitrogen by a similar amount. Non-ammoniacal nitrogen, and hence crude protein thus remain at approximately steady concentrations within the liquor. This could indicate steady biomass levels within the liquor, or indeed a steady rate of nitrogen (protein) breakdown and assimilation within the solid phase.

The maximum liquor ammoniacal nitrogen concentration was 699ppm. This is well below the level at which any ammonia toxicity might occur (McCarty, 1964). Higher levels of ammoniacal nitrogen could be tolerated; van Velsen (1979) has shown that methanogenic bacteria are readily able to adapt to ammoniacal nitrogen concentrations of up to 4990mg/litre, whereas concentrations above 3000 mg/litre have formerly been quoted as toxic (McCarty and McKinney, 1961).

The nitrogen content of the solid phase was shown to decrease slightly during the course of digestion. Over 70 day digestion periods, ammoniacal nitrogen decreased by an average of 436 ppm from an initial level of 1676 ppm. Total nitrogen

decreased by 576 ppm from an initial level of 5208 ppm. These in part are accounted for by the increased liquor ammoniacal nitrogen concentration. Some de-amination of proteins is likely to have occurred, along with a loss of ammonia (NH_3) as vapour into the gaseous phase. It is also possible that some denitrification may have occurred (Ghosh et al, 1976)

3.4.8 Total and Volatile Solids Content of the Liquor Phase

During the course of digestion, the volatile solids content of the liquor dropped from an initial level of 71.49% to a lower steady level of approximately 28% in the first 20 days of operation (Fig.3.7). Throughout the course of digestion the total solids content of the liquor remained approximately steady, fluctuating in an apparently random manner between 1.3 and 1.7% (average 1.52%) (Fig.3.7). The initial inoculum contained an average 30.3g of volatile solids, whereas the 'steady state' liquor contained only 12.7g of volatile solids. Thus the organic fraction of the liquor was decreased by almost 60%. The lower 'steady' level of volatile solids concentration coincided with the decrease in daily gas production shown in Figure 3.1.

The decreased level of biodegradable material within the liquor phase is possibly a function of the availability of easily degradable material in the solid phase. As digestion proceeds, easily degardable substrates become progressively scarcer, and the material carried over into the liquor will contain a greater proportion of lignin and salts which are non-biodegradable (Zeikus, 1980a).

It is believed that the drop in gas production after 20 days of operation was due to the lower availability of easily biodegradable substrates. After this time digestion proceded at a steady but declining rate, as more recalcitrant substrates such as cellulose are degraded (Jewell et al, 1979).

3.5 Conclusions of Preliminary Programme

1) The concept of laboratory scale anaerobic digestion in percolating packed bed digesters was shown to work successfully. No blockages of either the packed bed or recirculation system occurred in over 250 days of digester operation.

2) Increases in volatile fatty acid concentrations during start up were transient and no souring of digesters occurred. Ammoniacal nitrogen did not approach toxic levels at any time during the course of digestion.

3) The rapid recovery of the system after an influx of oxygen might be of considerable importance if routine or enforced maintenance work had to be conducted on operational digesters.

4) Gas yields obtained were largely comparable to the findings of other workers using similar substrates. It was anticipated that the figures could be considerably improved upon when operating conditions were optimised, and this is shown to be so in Chapter 4.

5) The volatile solids loss of 31.2% and cellulose loss of 57.2% are also encouraging. The solid substrate was reduced in volume by around 30% during the course of digestion. This will lead to considerable space savings if the material is to be stored before final disposal and will also reduce transport costs.

6) Gas production decreased rapidly after 20 days of operation, and this may prove to be important when optimum retention times are considered.

7) Approximately 88% of the total nitrogen input to the digester is retained, and hence the majority of its fertiliser value. Of this loss, 30% is present as increased liquor nitrogen concentrations.

8) Odour levels were diminished, in particular the distinct ammoniacal odour of the initial solid.

Figure 3.1 Daily Gas Production

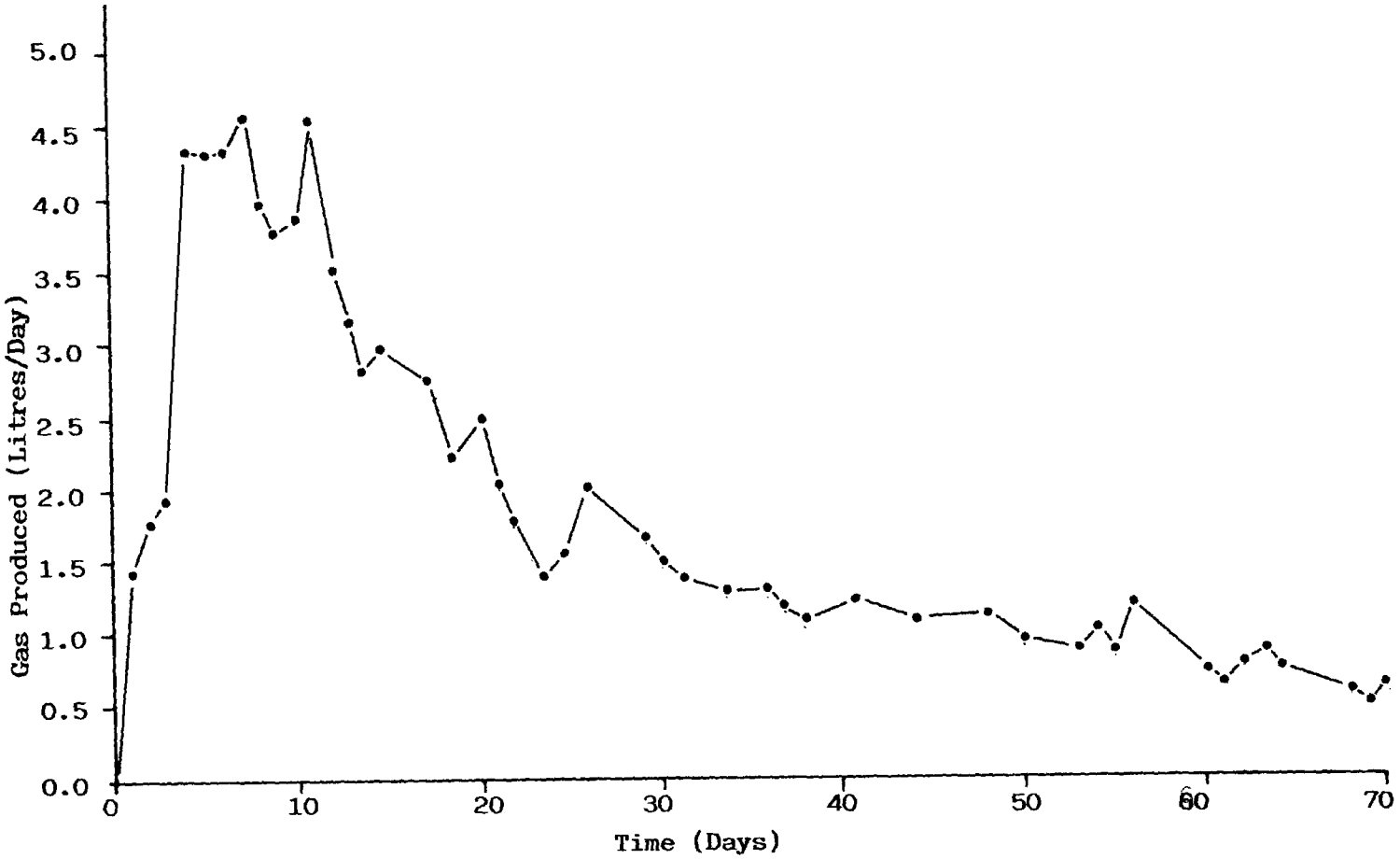


Figure 3.2 Gas Yield

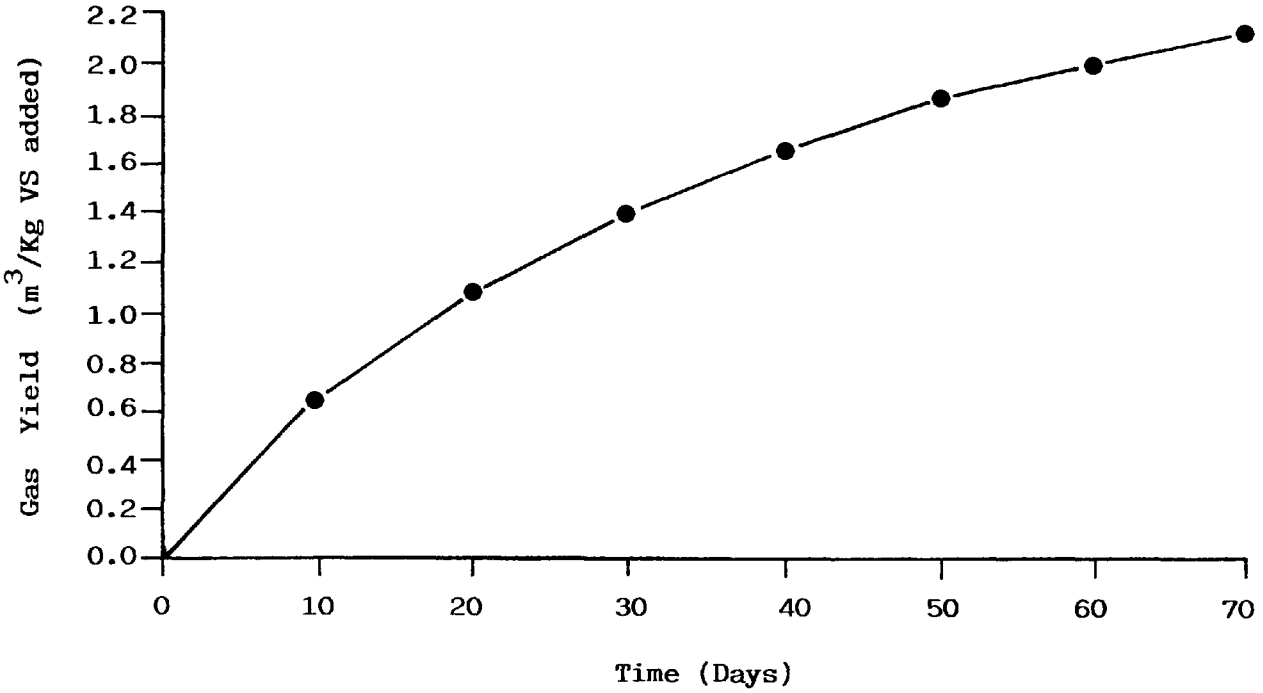


Figure 3.3 Biogas Methane Content

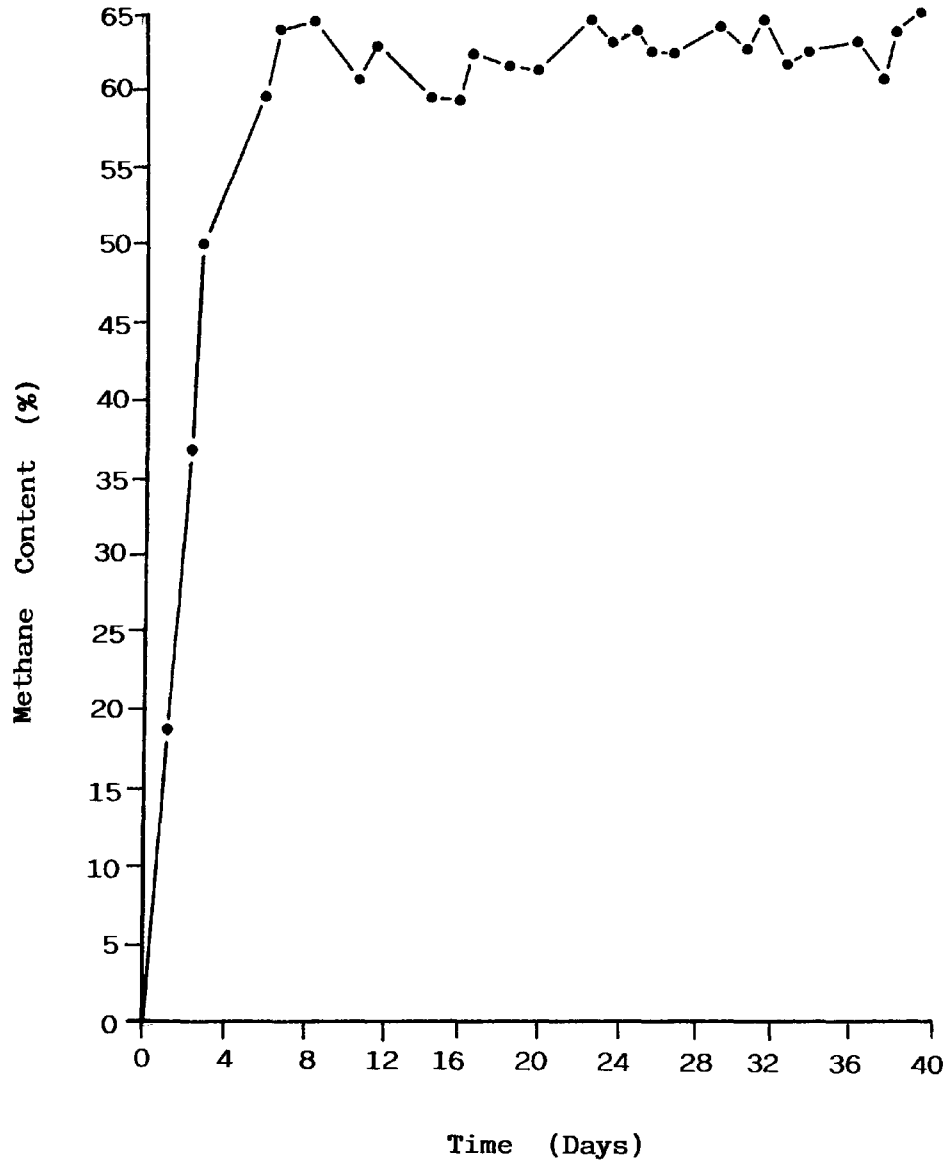


Figure 3.4 Liquor Volatile Fatty Acid Concentration

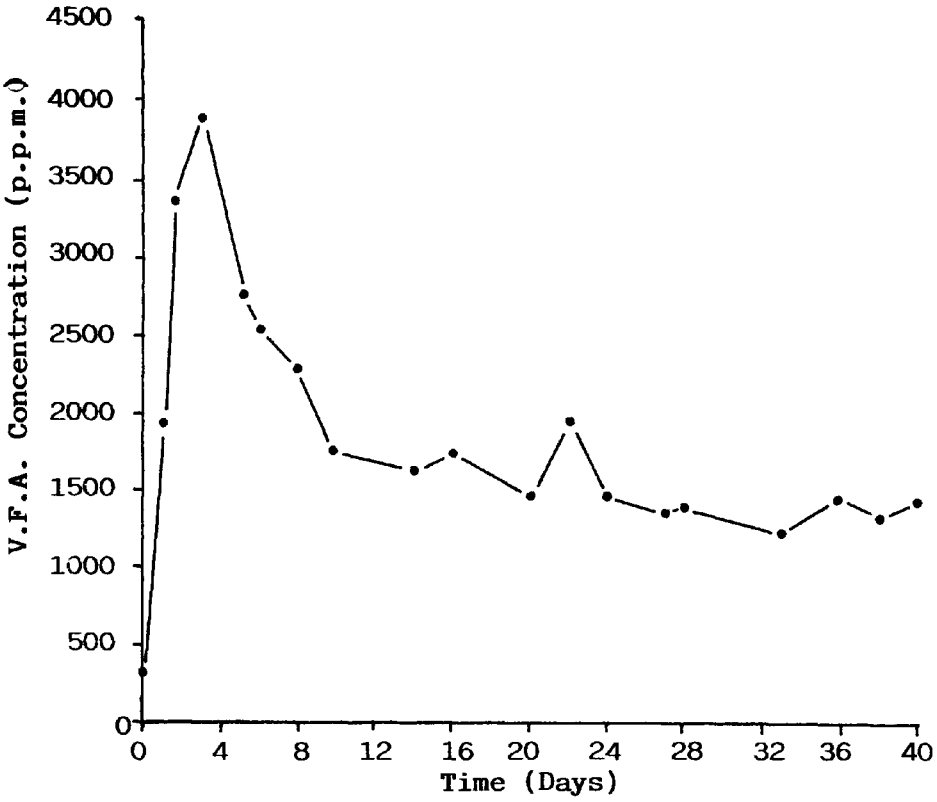


Figure 3.5 Liquor pH

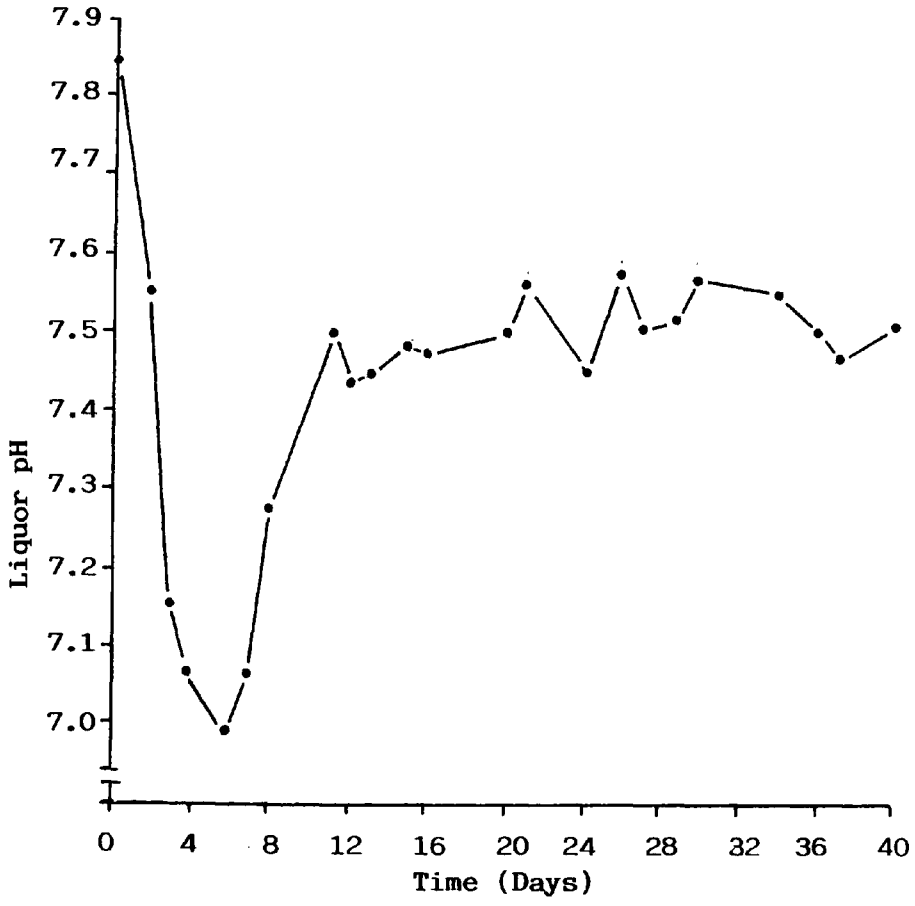


Figure 3.6 Increase in Liquor Nitrogen Concentration with Time

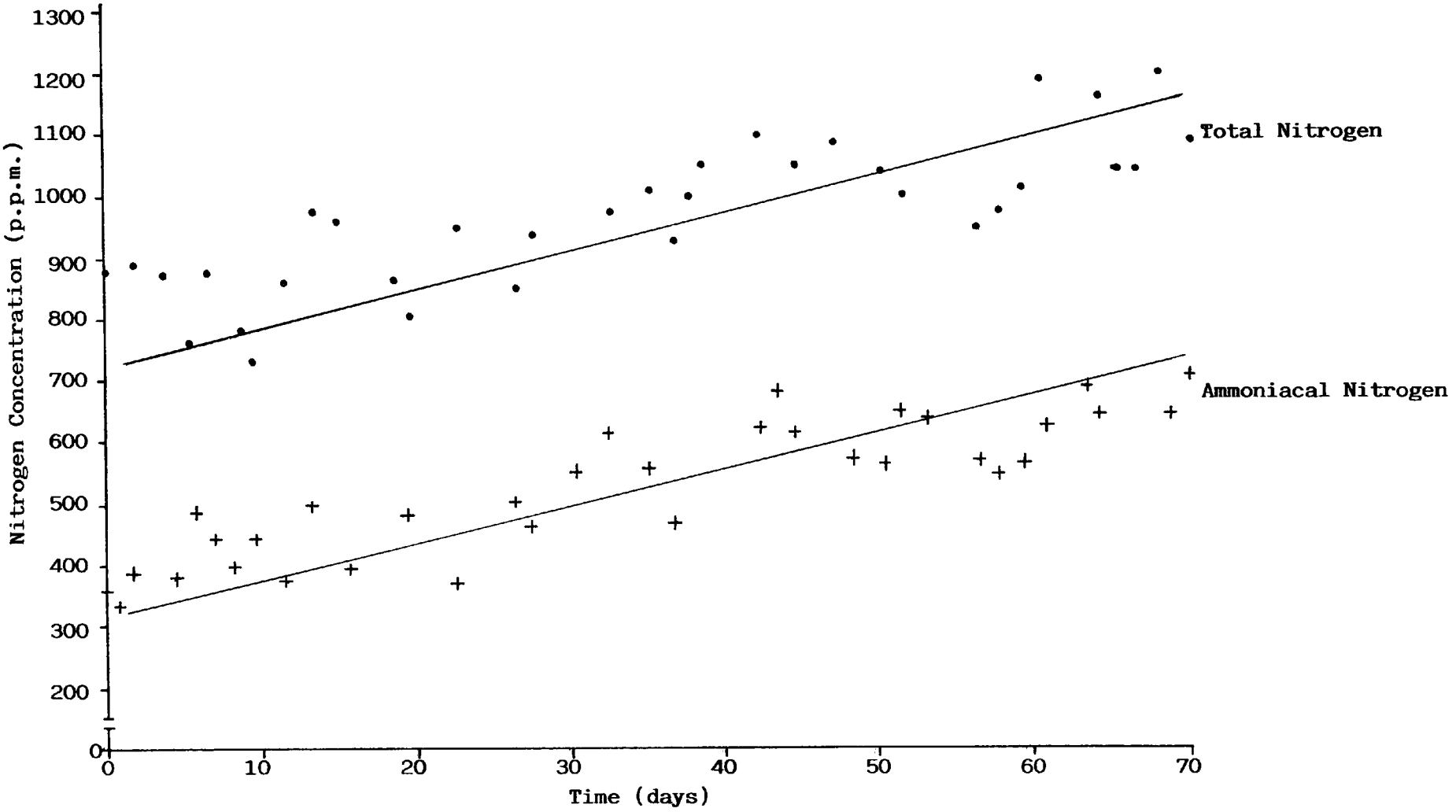
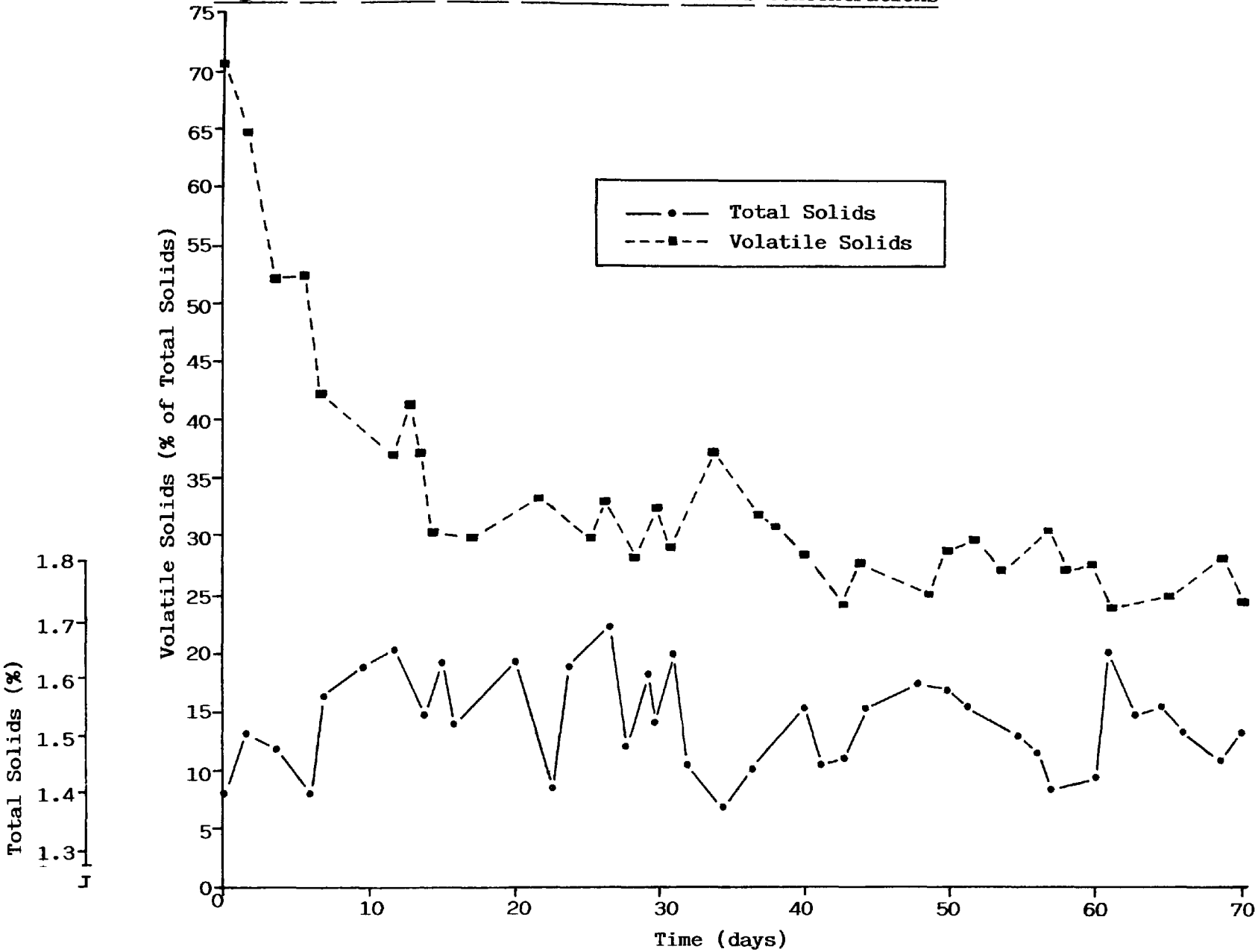


Figure 3.7 Liquor Total and Volatile Solids Concentrations



CHAPTER 4

OPTIMISATION - BATCH PROCESS

4.1 Introduction

The results presented in Chapter 3 provide the basis for the optimisation of biogas production from percolating packed bed digesters. The system used allows a number of operational parameters to be easily and independently varied. In addition to temperature it was believed that liquor recirculation rate, solid : liquid ratio and the digester bed height would influence biogas production from this type of digester. Optimisation of the process to increase biogas production is desirable, since energy saving brought about by the use of biogas as an alternative fuel source is likely to be the main factor to be considered when the economics of a full scale system are evaluated.

4.2 Digester Operation

Basic operation of the digesters has been previously described. In this section the experimental programme is expanded to include a study of bed height as an operating variable. In this program of research one operational parameter was varied independently of the others, the operational parameters considered being temperature, liquor recirculation rate, solid : liquid ratio and bed height. The specific operating conditions for each variable are discussed in the following sections. All experiments were

conducted in duplicate over periods of 40 days except those relating to bed height where a 30 day operational period was used. All experimental results presented are averages of two duplicate runs.

4.3 Substrate Composition

The same substrate was used in experiments relating to temperature, solid : liquid ratio and liquor recirculation rate and the analyses are shown in Table 4.1. The somewhat different composition of substrate used when investigating the effects of bed height is shown in Table 4.2. In particular there is a greater percentage wet weight of cellulose and lower total nitrogen content, thus leading to a higher Carbon : Nitrogen ratio, closer to the optima found by Hills (1979).

Table 4.1 Substrate Composition used in the investigation of temperature, recirculation rate and solid : liquid ratio

Total Solids	21.48%
Volatile Solids	18.38%
Total Nitrogen	5938ppm
Ammoniacal Nitrogen	1983ppm
Volatile Fatty Acids	3017ppm
Cellulose	13.03% wet weight
non-lignin C:N ratio	19:1
Adenosine triphosphate	0.03×10^{-6} mol/kg

Table 4.2 Substrate Composition used in the investigation of bed height

Total Solids	27.73%
Volatile Solids	20.02%
Total Nitrogen	4165ppm
Ammoniacal Nitrogen	1120ppm
Volatile Fatty Acids	2550ppm
Cellulose	14.89% wet weight
non-lignin C:N ratio	21:1
Adenosine triphosphate	0.02×10^{-6} mol/kg

4.4 Results

4.4.1 Temperature as an Operating Variable

4.4.1.1 Operating Conditions

The digesters were operated as single units, using a solid : liquid ratio of 2:1 (6kg of solid substrate : 3 litres of liquor), with a liquor recirculation rate of 3 litres/hr and a bed height of 0.51 metres. The digesters were operated at three temperatures within the mesophilic range, namely 25, 30 and 35°C.

4.4.1.2 Gas Yield

Under the conditions chosen an almost linear increase in gas yield with increasing temperature was obtained (Fig.4.1), and results are given in Table 4.3.

This trend is in close agreement with the results of van den Berg and Kennedy (1982) and is as would be expected with increased temperature, when bacterial reproduction and metabolic rates are increased, enzyme catalysed reactions increasing two fold with a 10°C increase in temperature. The results obtained are comparable to those of previous workers, for example Hills (1980b) who obtained a gas yield of 0.19m^3/kg VS added from a combination of dairy manure and barley straw. The yields also show a significant increase (approximately 40% at 30°C) from those previously obtained. This will be partly due to the substrate composition, and the high solids : liquid ratio used in these experiments. The increase in gas yield per kg volatile solids destroyed is possibly a result of different groups of bacteria which degrade different substrates becoming proportionately more important as temperature was increased.

4.4.1.3 Gas Production

The results of daily gas production (Fig.4.2) show trends similar to those previously obtained but with a number of significant variations. For example at 30°C the initial increase in daily gas production was slower than that shown in Fig.3.1, with the maximum 'steady' level being reached after 11 days operation,

Table 4.3 Effect of Temperature on Gas Yield

(All results presented are averages)

Temperature of Operation	Weight of V.S. added	Weight of V.S. destroyed	Total Gas Production after 40 days	Gas Yield m^3/kg V.S. added	Gas Yield m^3/kg V.S. destroyed
25°C	1.103kg	0.366kg	189.7 litres	0.172	0.518
30°C	1.103kg	0.433kg	259.2 litres	0.235	0.589
35°C	1.103kg	0.528kg	336.4 litres	0.305	0.637

and remained at this level for a further 12 days. This can be accounted for by the increased solids loading and solid : liquid ratio which led to an increase in concentration of liquor constituents.

At 35°C there was a more rapid rise in the rate of gas production than at 30°C, reaching its maximum after 7 days and maintaining this rate for a further 9 days. The maximum rate of gas production was significantly greater than at 30°C. The rate of gas production then decreased very rapidly, reaching a lower level of between 3 and 4 litres/day after 24 days of operation. This rate is lower than that at either 25 or 30°C and is possibly due to substrate limitations brought about by the high rate of substrate usage during the early part of digestion.

At 25°C there was a long phase of reduced gas production compared with the two high temperatures, maximum production rates occurring only between days 13 and 17. The rate then decreased to approximately 5 litres/day and was maintained at this level. It is probable that a slower but more even rate of gas production would be obtained at lower temperatures due to the slow rate of substrate degradation, substrate limitation not occurring even after 40 days of operation. This would be expected of bacterial reaction and growth rates in relation to temperature.

4.4.1.4 Liquor Volatile Fatty Acid Concentration

Previous work (Hall *et al*, 1985b) has shown that during start-up of percolating packed bed digesters the increased volatile fatty acid concentration may cause the depression of daily gas

production due to the inhibition of methanogenesis. Liquor VFA concentration was shown to rise rapidly in all cases, reaching a concentration of around 5000ppm after 3 days (Fig.4.3). The high VFA levels were maintained for different periods depending on the temperature of operation, and was reflected in proportionately longer delays in reaching maximum gas production with decreasing temperature. For example, at 25°C high levels of VFA's were maintained for 10 days whilst at 35°C high levels were maintained for only 4 days.

The increased period of high VFA concentration at lower temperatures may be indicative of poorer performance of the methanogenic bacteria compared with the acid producing bacteria at lower temperatures, it thus taking longer for the methanogens to degrade the back-log of acetate production. This is borne out by the methane content of the biogas at various temperatures.

4.4.1.5 Gas Composition

The methane content of the biogas is shown in Figure 4.4. Maximum methane content was reached after 7 days at 35°C, 10 days at 30°C and 14 days at 25°C. This was in close agreement with the results of Ehrig (1983) who found it took 2.5 times as long to reach maximal methane production at 20°C compared to 30°C. There was very little difference in the steady biogas composition at the different temperatures, though some differences might be expected due to the increased solubility of carbon dioxide at lower temperatures (Rozzi *et al*, 1983), though this is possibly counteracted by the poorer performance of methanogens at lower

temperatures.

4.4.1.6 Solids Losses

The results obtained are shown in Table 4.4. Solids losses increased with temperature as would be expected from the previous results and are substantial losses over a relatively short period of time. Also noted were volume reduction in the digested waste in the order of 30%. Finck and Goma (1981) operating digesters at 35°C obtained total solids reductions of 29.7% and volatile solids reductions of 34.9%.

4.4.1.7 Variation in Liquor Composition

Variation in liquor composition showed trends similar to those obtained in the initial feasibility study. pH was initially depressed, and for progressively longer periods at lower temperatures.

Total solids remained at similar levels throughout the course of digestion (average 1.91% ± 0.23) and was apparently unaffected by temperature. Volatile solids decreased from an initial level of 71% to around 55% in all cases after 40 days. This concentration was higher than in the initial feasibility study and was probably due to greater substrate availability at higher solids loadings. There was no significant variation in volatile solids content with temperature. Ammoniacal and total nitrogen were again shown to increase during the course of digestion, to similar extents independent of temperature. The

Table 4.4 Effect of Temperature on Solids Losses
(All results shown are averages)

Initial Weight of Total Solids : 1.289kg

Initial Weight of Volatile Solids : 1.103kg

Initial Weight of Cellulose : 0.782kg

Wt. of TS remaining at 25°C after 40 days : 0.977kg

Wt. of TS remaining at 30°C after 40 days : 0.856kg

Wt. of TS remaining at 35°C after 40 days : 0.805kg

Wt. of VS remaining at 25°C after 40 days : 0.736kg

Wt. of VS remaining at 30°C after 40 days : 0.669kg

Wt. of VS remaining at 35°C after 40 days : 0.575kg

Wt. of cellulose remaining at 25°C after 40 days : 0.503kg

Wt. of cellulose remaining at 30°C after 40 days : 0.374kg

Wt. of cellulose remaining at 35°C after 40 days : 0.276kg

Total solids destroyed at 25°C : 24.18%

Volatile solids destroyed at 25°C : 33.21%

Cellulose destroyed at 25°C : 35.62%

Total solids destroyed at 30°C : 33.59%

Volatile solids destroyed at 30°C : 39.27%

Cellulose destroyed at 30°C : 52.15%

Total solids destroyed at 35°C : 37.53%

Volatile solids destroyed at 35°C : 47.84%

Cellulose destroyed at 35°C : 64.71%

increase was greater than in the initial studies however both total and ammoniacal nitrogen showed increases of 950ppm, thus giving a constant level of crude protein (non-ammoniacal nitrogen) in the liquor. Maximum liquor ammoniacal nitrogen concentration was 1343ppm, well below the toxic levels quoted by McCarty (1964). The higher levels of nitrogenous material in the liquor phase was caused by the lower dilution factor than in the initial study at higher solids : liquid ratios. The increase in liquor nitrogen was again due to the loss of on average 584ppm of ammoniacal nitrogen and 802ppm of non-ammonia nitrogen from the solid phase. A small percentage of initial nitrogenous material was again lost from the system, which was apparently temperature independent, which might indicate the loss of ammonia from the system as vapour to be negligible.

4.4.1.8 Adenosine 5'triphosphate Concentrations

The results showed substantial differences in ATP concentration in the solid phase at the end of the digestion period in relation to temperature. At 35°C ATP concentration was 15.8 x 10^{-6} moles/kg wet weight and at 25°C was 11.5 x 10^{-6} moles/kg wet weight while average liquor concentrations were very similar being 1.37 x 10^{-6} M and 1.35 x 10^{-6} M respectively. These trends indicate that at higher temperatures there was greater colonisation of the solid bed as might be expected with increased bacterial reproduction rates. The similar liquor concentrations however were possibly caused by a finite number of bacteria being able to survive unattached in the liquor due to the limitation of a key

nutrient or growth factor.

4.4.1.9 General Observations

The advantage of greater gas production at 35°C may be offset by the heat input required to maintain this temperature. The greater the operating temperature is above ambient temperature, the more biogas will be required to maintain digester temperature. It is possible therefore that a temperature lower than 35°C will be optimal for net energy production in a full scale unit (Hawkes & Horton, 1981).

However it may be argued that percolating packed bed digesters may have a higher optimal temperature of operation than conventional digesters. High solids digesters will have a smaller surface area for heat losses to occur, and thus maintenance of temperature will require lower heat input. In addition the substrate used will have a smaller volume and lower water content and will thus require a lower heat input to maintain operating temperature.

4.4.2 Recirculation Rate as an Operating Variable

At the outset, little was known of the effects of recirculation rate. It was known that the bed would operate without fluid recirculation at least for a limited period of time, but that recirculation would increase gas production (Petersen, 1981), and would be necessary in future work involving the linking of digesters in series to form a semi-continuous system.

If the system is considered to be similar to a fixed film process with bacteria attached to the bed, then the rate of recirculation must be below the level at which bacteria become detached from the supporting matrix. It has been suggested (Colleran, 1985) that the daily flow through a fixed film reactor should not exceed eight times the digester volume, which under the conditions used in these experiments would be approximately 2 litres/hr.

4.4.2.1 Operating Conditions

The digesters were operated as single units using a solid : liquid ratio of 1:1 (3kg solid substrate, 3 litres liquor), operating at 30°C with a bed height of 0.26 metres. Experiments were conducted at four recirculation rates; 0, 0.6, 3.0 and 15.0 litres/hr. For zero recirculation, inoculum was added and recirculated for seven days before being drained off.

4.4.2.2 Gas Yield

The effect of liquor recirculation rate on gas yield was shown to be very small at all recirculation rates used (Fig.4.5). The results are shown in Table 4.5.

The depression of gas yield at the highest recirculation rate (15.0 litres/hr) is probably due to the detachment of attached bacteria, and is indicated by the relatively high dry matter content of the liquor (Table 4.7). The yield obtained however is only 8.54% less than the maximum obtained at a recirculation rate of 0.6 litres/hr. This might indicate particularly tenacious attachment of bacteria to straw fibres, or that the flow of liquor through the bed is so dispersed that severe detachment of bacteria does not occur even at recirculation rates over seven times greater than that at which detachment might be expected to occur in conventional fixed film reactors.

4.4.2.3 Gas Production

Due to the similarities in gas yield obtained with different recirculation rates a clearer picture was obtained if the gas production was considered over separate 10 day periods during each run, the results being expressed in units of litres per kg per day ($1.kg^{-1}.day^{-1}$). The results are shown in table 4.6)

Table 4.5 Effect of Recirculation Rate on Gas Yield

Average Weight of Volatile Solids Added : 0.551kg

Recirculation Rate (litres/hr.)	0	1.6	3.0	15.0
Cumulative Gas Production after 30 days (litres)	76.1	77.7	76.6	73.9
Cumulative Gas Production after 40 days (litres)	87.8	90.4	89.8	82.7
Gas Yield (m^3/kg VS added) after 30 days	0.138	0.141	0.139	0.134
Gas Yield (m^3/kg VS added) after 40 days	0.159	0.164	0.163	0.150

Table 4.6 Average Gas Production

Recirculation Rate (litres/hr)	0	0.6	3.0	15.0
Average gas production ($l.kg^{-1}.day^{-1}$) for				
period 0 - 10 days	0.85	0.77	0.94	0.73
10 - 20 days	1.26	1.20	1.17	1.03
20 - 30 days	0.66	0.81	0.73	0.70
30 - 40 days	0.30	0.53	0.56	0.51

It can be seen (Fig.4.6) that the gas yield at zero liquor recirculation was initially close to that obtained at rates of 0.6 and 3.0 litres/hr. However the rate of gas production decreased much more rapidly after around 20 days of operation with no recirculation. Thus, although the digester was able to operate normally for around 20 days after start up, its performance then deteriorated rapidly. This was most probably due to poorer inoculation throughout the bed as a whole, causing certain areas of the bed to be undigested. High levels of volatile fatty acids were not apparent in the solid phase and it is therefore unlikely that high levels of toxicants are responsible for the rapid decrease in gas production at zero recirculation rate. Similar results for fixed film reactors were obtained by Dugg and Kennedy (1983) who found recirculation (provided it was present) had little effect on the start-up or steady state operation of fixed film reactors.

4.4.2.4 Gas Composition

Methane content of the biogas reached its maximum level at all recirculation rates after between 5 and 6 days of operation. The maximum methane content did however vary with recirculation rate in a similar manner to gas yield. At zero recirculation methane accounted for 59.1% (±1.1%) of the biogas, while at a recirculation rate of 0.6 litre/hr the composition was 63.5% (±2.5%). With the recirculation rate further increased to 3.0 litres/hr the methane content dropped to 62.4% (±2.1%) and a further increase in rate to 15.0 litres/hr led to the methane content decreasing to 60.3% (±1.9%). The reasons for this are not clear.

At high recirculation rates lower methane content may be caused by the higher level of bacterial detchment, with a proportionally greater detachment of methanogenic bacteria, thus removing them from areas of VFA production. At zero recirculation the lowering of methane content may be a result of reduced substrate availability to the methanogenic bacteria caused by poorer transfer of metabolites through the bed.

4.4.2.5 Solids Loss

Recirculation rate was shown to have little effect on the percentage solids losses, with a rate of 3.0 litres/hr leading to marginally greater solids losses (Fig.4.7). It may be argued therefore that due to the small differences in gas yield at recirculation rate of 0.6 and 3.0 litres/hr, the latter is the

optimum recirculation rate.

The solids losses obtained in this study were marginally greater than those obtained in the initial feasibility study when the same operating conditions were used. This was most probably due to different feedstock composition, in particular its Carbon: Nitrogen ratio, which in the initial study was 17:1 compared with a ratio 19:1 in this study.

4.4.2.6 Liquor Compositional Changes

Compositional changes in the liquor showed similar trends to those previously recorded. Volatile Fatty Acid concentrations were closely similar at recirculation rates of 0.6, 3.0 and 15.0 litres/hr. There was an initial increase over 4 days to concentrations between 2890 and 4270ppm, followed by a rapid decrease to a steady state level of 1380 (±167)ppm.

The pH profile was also apparently independent of the recirculation rate used, falling to 7.16 (±0.11) after 6 days of operation, then increasing to an approximately steady level of 7.58 (±0.23) after 12 days.

The dry matter content of the liquor was found to vary with recirculation rate:

Table 4.7 Variation in Liquor Dry Matter Content

Recirculation rate (litres/hr)	Total solids (%)
0.6	1.53 (±0.21)
3.0	1.55 (±0.15)
15.0	1.73 (±0.18)

The increase in dry matter with increasing recirculation rate was most probably caused by increased leaching of soluble components and attached bacteria from the bed. This is indeed indicated by the volatile solids content of the liquor.

At recirculation rates of both 0.6 and 3.0 litres/hr the liquor volatile solids (as a percentage of T.S) reaches a minimum level of around 30% after between 20 and 23 days of operation regardless of recirculation rate. At a recirculation rate of 15 litres/hr the same VS content was reached only after 30 days of operation. Thus at higher flow rates more biodegradable material is leached from the solid matrix.

Liquor ammoniacal nitrogen and total nitrogen again increased throughout the course of digestion, in the manner shown in Table 4.8.

The relative increases in liquor nitrogenous material was again a function of increasing recirculation rate, and is probably related to the rate of leaching of soluble material from the solid matrix. It was noticeable from the results a small increase in non-ammoniacal nitrogen in the liquor, not apparent in the initial feasibility study (possibly due to experimental error).

Table 4.8 Effect of Recirculation Rate on Liquor Nitrogen Concentrations

Recirculation Rate ($l.hr^{-1}$)	0.6	3.0	15.0
Average Initial Liquor Ammoniacal Nitrogen Concentration (ppm)	356	356	356
Average Initial Liquor Total Nitrogen Concentration (ppm)	866	866	866
Average Final Liquor Ammoniacal Nitrogen Concentration (ppm)	559	643	718
Average Final Liquor Total Nitrogen Concentration (ppm)	1141	1184	1255
Average increase in Liquor Ammoniacal Nitrogen Concentration (ppm)	203	287	362
Average increase in Liquor Total Nitrogen Concentration (ppm)	275	318	389

The increase was similar at all recirculation rates and was possibly caused by an increase of recalcitrant nitrogenous material, such as lignin masked proteins, during the course of digestion. Maximum ammoniacal nitrogen concentration in the liquor was 749ppm and was thus non-toxic.

The increases in liquor nitrogenous material are partly accounted for by the loss of such material from the solid phase. When the solid phases were analysed after digestion the following losses (Table 4.9) were found to have occurred, by comparison with the original levels of nitrogenous material shown in Table 4.1.

Table 4.9 Loss of Nitrogenous Material from the Solid Phase

Recirculation rate (litres/hr)	0	0.6	3.0	15.0
Ammoniacal nitrogen loss (ppm)	414	503	437	594
Non-ammoniacal nitrogen loss (ppm)	270	581	563	639

At zero recirculation the loss of non-ammoniacal nitrogen was very low, and was presumably a function of low removal of soluble material from the bed when there was no liquor recirculation.

When recirculation is present, its rate does not appear to affect the loss of nitrogenous material.

4.4.2.7 General Observations

It would appear from these experiments that recirculation rate has only a marginal effect on gas production providing it was not excessively high or low. The gas yield obtained at zero recirculation rate was close to the maximum, and was found to preserve the nitrogen content of the feed, but a distinct tail off in gas production during the latter stages of digestion indicates poor colonisation of the bed, or poor substrate transfer within it. In addition, the linking of digesters in series would not be possible. High recirculation rates appear to depress gas production probably due to the detachment of bacteria from the solid matrix. Marginally greater solids losses were obtained at a recirculation rate of 3 litres/hr, this rate was therefore used in all future experimental work.

4.4.3 Solid : Liquid Ratio as an Operating Variable

4.4.3.1 Operating Conditions

In this series of experiments the weight of solid material and liquor volume initially added were varied and their effects determined. All experiments were conducted at 30°C and a liquor recirculation rate of 3 litres/hr. The bed height used varied proportionately with the weight of solid material added, a 6kg solids loading corresponding to a bed height of 0.51 metres and a 3kg solids loading being equivalent to a bed height of 0.26 metres.

4.4.3.2 Gas Production and Yield

The gas yield from percolating packed bed digesters was found to be greatly influenced by the solid : liquid ratio (Fig.4.8), with a variation in yield of up to 36% over the range studied. The rate of gas production in terms of litres per kg per day as described in section 4.4.2.3 over 10 day periods was consequently affected in the same manner. The considerable variation in rates of gas production and gas yield (shown below) indicate the importance of solid : liquid ratio on the digestion process.

Table 4.10 Effect of Solid : Liquid ratio on Gas Production

Ratio	1:1	1.3:1	2:1	2:1	4:1
Solids added (kg)	3	4	3	6	6
Liquor added (litres)	3	3	1.5	3	1.5
Bed height (metres)	0.26	0.35	0.26	0.51	0.51
Weight of V.S. added (kg)	0.551	0.735	0.551	1.103	1.103
Average Cumulative Gas Production (litres)	89.8	138.9	119.0	259.2	164.3
Gas Yield (m^3/kg VS added)	0.163	0.189	0.216	0.235	0.149
Average Gas Production ($l.kg^{-1}.d^{-1}$) for period					
0 - 10 days	0.94	0.93	0.80	0.87	0.46
10 - 20 days	1.17	1.29	1.63	1.77	0.93
20 - 30 days	0.73	1.32	1.50	1.49	0.90
30 - 40 days	0.56	0.95	0.67	1.29	0.74

Investigations showed that at a solid : liquid ratio of 2:1, at a 6 kg solids loading approximately 1.2 litres liquor was in the recirculation system and thus 1.8 litres was trapped within the solid matrix. While at a solid : liquid ratio of 1:1 at a 3 kg solid loading approximately 2.1 litres of liquor was in the recirculation system. Thus liquor hold-up within the bed was approximately 0.3 l/kg. Of this 0.3 litres part will be permanently within the bed, hydrating the solid matrix, and the remainder will be held within the bed, but flowing through it. From these considerations, at the highest ratio (4:1) with 6 kg of solids in the bed, 1.8 litres of liquor could be held up within the bed. From Table 4.10 it can been seen that only 1.5 litres was being

recirculated in accordance with the experimental programme.

The results obtained here were similar to those obtained in Section 4.4 but the evidence here indicates more clearly that if liquor recirculation was too low then the rate of digestion decreases.

This set of experiments illustrated that contact time between the solid and liquid is a significant factor in digestion rate, this may be due to poor distribution of micro-organisms throughout the matrix at low flow rates and also poor mass transfer of substrate and metabolites at low liquid flow. Conversely, at high flow rates the substrate may be transferred through the matrix too quickly.

A low solid : liquid ratio will lead to the greatest dilution of metabolites, and will thus reduce their availability to attached bacteria.

Thus solid : liquid ratio is critical to gas production, a ratio of 4:1 depresses gas yield by 36.6% compared to a 2:1 ratio, and similarly a ratio of 1:1 depressed the gas yield by 24.5% compared to a 2:1 ratio, all other conditions being the same.

4.4.3.3 Effects of Solid : Liquid ratio on VFA concentration and pH

The concentration of VFA's in the liquor show trends similar to those previously obtained (Section 4.4.1.4) and are shown in Fig.4.9, with the concentration increasing to a high initial level and then falling to a lower steady level which is maintained throughout the course of digestion.

The lower steady level of volatile fatty acid concentration, appeared to dictate the overall rate of gas production and gas yield. The average steady VFA concentrations are shown below.

Solid : liquid Ratio	VFA concentration
1:1	1484 ppm
1.3:1	1870 ppm
2:1	2135 ppm
4:1	1261 ppm

Volatile fatty acids (directly acetate) are the main precursors of methane in anaerobic digestion, and an increased concentration providing it does not reach inhibitory levels will enhance gas production (Stafford, 1982). Thus an increased solid : liquid ratio will lower the dilution of metabolites in the liquor causing increased VFA concentration and therefore increased gas yield.

The low liquor VFA concentration at a solid : liquid ratio of 4:1, which contributed to poor gas production, was possibly a result of undigested areas of the solid matrix not releasing VFA's. This does not however preclude the existence of areas within the solid matrix having inhibitory concentrations of VFA's as they would not be detected during final analysis of the bed.

The low rates of gas production over the first 10 days of operation (Table 4.10) at a ratio of 2:1 compared to a solid : liquid ratio of 1:1 can be accounted for by the higher

concentration of volatile fatty acids, during start-up and that high VFA concentrations are maintained for a longer period (Fig.4.9) with increased solid : liquid ratio, thus inhibiting gas production.

pH follows the trends associated with changes in the concentration of volatile fatty acids. The relatively small differences in the lower steady concentration of VFA's are not reflected in the pH (7.56 ±0.21 in all cases), probably due to the high buffering capacity of the system, alkalinities of up to 8000 mg/l of calcium carbonate being recorded.

4.4.3.4 Gas Composition

At solid : liquid ratios of 1:1, 1.3:1 and 2:1 the methane content of the biogas reached a maximum level after between 6 and 10 days of operation, and was found to be dependent on length of time volatile fatty acids were maintained at high inhibitory levels, causing an inhibition of methanogenic bacteria by the low pH and high redox potential developed.

At a solids : liquid ratio of 4:1 maximum methane content of the biogas was obtained after 18 days of operation, and was then maintained at a level of 60.5%. This would seem to show that toxic levels of VFA's do develop trapped within specific areas of the bed, as liquor VFA's were not at inhibitory levels for this extended period and were therefore not the cause of inhibited methane production.

4.4.3.5 Solids Losses

The solids losses obtained (Table 4.11) were in line with those expected from the results of the gas yields previously discussed, clearly showing (Fig.4.10a) a solids to liquid ratio of 2:1 is optimum with a slightly improved level of solids destruction at a 6kg solids loading, compared with a 3kg solids loading. This was a result of increased bed height and is discussed in Section 4.4.4.

4.4.3.6 Liquor Composition

The total solids content of the liquor showed a general trend of small random fluctuations, around a steady average value and was shown to increase (Fig.4.10b) with increased solid : liquid ratio. This was most probably caused by the lower dilution of liquor constituents at higher solid-liquid ratios.

The volatile solids content of the liquor remained at higher levels with increasing solid : liquid ratio. At a ratio of 1:1 VS reached a minimum level of around 30% after 25 days of operation, whereas at a ratio of 2:1 volatile solids concentrations of between 40 and 55% were observed after 40 days of operation. This was possibly caused by increased contact time between the solid matrix and the liquor at higher solid : liquid ratios.

Liquor ammoniacal and total nitrogen concentration showed similar trends to those previously described, with the solid : liquid ratio having no detectable influence. Ammoniacal nitrogen did not approach inhibitory levels in either the solid phase or the

Table 4.11 Effect of Solid : Liquid Ratio on Solids Losses

Ratio	1:1	1.3:1	2:1	2:1	4:1
Solids added (kg)	3	4	3	6	6
Liquor added (l)	3	3	1.5	3	3
Weight T.S. added (kg)	0.644	0.859	0.644	1.289	1.289
Weight V.S. added (kg)	0.551	0.735	0.351	1.103	1.103
Weight Cellulose added (kg)	0.391	0.521	0.391	0.782	0.782
Weight T.S. remaining (kg)	0.497	0.654	0.462	0.856	1.012
Weight V.S. remaining (kg)	0.409	0.532	0.366	0.669	0.826
Weight Cellulose remaining(kg)	0.252	0.332	0.215	0.374	0.538
T.S. Loss (%)	22.9	23.9	28.3	33.6	21.5
V.S. Loss (%)	25.8	27.6	33.7	39.3	25.1
Cellulose Loss (%)	35.6	36.3	45.1	52.2	31.2

liquor phase at a ratio of 4:1, being 1587 (±268) ppm and 1385 (±142) ppm respectively, at the end of the digestion period. It does not seem likely therefore that decreased gas production at this ratio can be attributed to inhibitory levels of ammoniacal nitrogen. However isolated pockets of high ammoniacal nitrogen could be present in the solid matrix leading to inhibition, though no evidence was found to substantiate this.

4.4.3.7 General Observations

Solid : liquid ratio is thus an important parameter in determining gas production from percolating packed bed digesters. It affects the liquid held within the bed and the concentration of metabolites within the liquor, leading to variations in gas yield and solids digestion. The results showed an optimum ratio of 2:1.

The improved performance with higher solids loadings at a ratio of 2:1 is probably a function of increased liquor residence time within the bed, and will be more fully discussed in the following section.

4.4.4 Bed Height as an Operating Variable

4.4.4.1 Operating Conditions

As previously described, digesters could be bolted vertically together, maintaining the digester diameter, but increasing bed height and therefore the weight of substrate used. A series of experiments was thus conducted to examine the effects of bed heights. A solid : liquid ratio of 2:1 was used and a liquor recirculation rate of 3 litres/hr. Due to difficulties in achieving set point temperature an operating temperature of 31.5 (±1.0)°C was used. The experiments utilised 6, 12, 18 and 25 kg of substrate corresponding to bed heights of 0.51, 1.02, 1.53 and 2.05 metres (see Fig.4.11 and Plate 4.1). In this configuration it was anticipated that any practical limitation on bed height would be elucidated. Experiments were conducted in duplicate for periods of 30 days.

4.4.4.2 Effects of Bed Height on Gas Production

If the gas yields (m^3/kg VS added) for the various bed heights are examined (Table 4.12), there appears to be little variation, as might be expected from digesters utilising the same solid : liquid ratio and operating at the same temperature. The results (Table 4.12) of average gas production (litres.kg^{-1}.day^{-1}) over 10 day periods show large variations in the relative rates of gas production for each 10 day period at the different bed heights.

If the cumulative gas production is now examined (Fig.4.12),

Table 4.12 Gas Yield and Production with Increasing Bed Heights

Bed Height (metres)	0.51	1.02	1.53	2.05
Average Weight of Volatile Solids added (kg)	1.201	2.402	3.602	5.005
Average Cumulative Gas Production (litres)	254.7	535.7	810.8	1001.0
Gas Yield (m^3/kg VS added)	0.212	0.223	0.225	0.200
Average Gas Production ($l.kg^{-1}.d^{-1}$) for periods				
0 - 10 days	0.94	1.20	1.42	1.33
10 - 20 days	1.60	1.86	1.98	1.44
20 - 30 days	1.81	1.40	1.25	1.24

the results for each bed height being corrected to that of a 0.51 metre bed (i.e. the gas production from a 1.53 metre bed being reduced by a factor of 3) allowing direct comparisons to be drawn, it can be seen that although the gas production from digesters having a bed height of 2.05 metres begins rapidly, the rate decreases from around day 17. Observation showed this to coincide with blocking of the bed. Similarly gas production from digesters of bed height 1.53 metres decreased rapidly after around 23 days of operation, again due to blockages occurring. No blockages occurred in digesters of 0.51 or 1.02 metres, even after 60 days of operation. The higher gas yield at a bed height of 0.51 metres than those previously obtained (Section 4.4.3), was a function of different substrate composition (Tables 4.1 and 4.2) and the temperature of operation (31.5°C compared with 30.0°Cin section 4.4.3).

Blocking of the bed was most probably caused by the loss of rigidity in the straw fibres, as their cellulose content was progressively degraded, thus compaction followed by blocking occurred.

It was noted that blocking of the bed occurred in digesters with a bed height of 1.53 metres at the base of the bed, and within the lower 25% of beds whose heights were 2.05 metres. Thus the maximum bed height at a reactor diameter of 0.18 metres and using this particular waste was less than 1.53 metres.

4.4.4.3 Liquor VFA Concentration

Liquor volatile fatty acid concentrations showed trends similar to those previously described, with an initial increase followed by a steady decline to a lower steady level. These are shown in Figure 4.13. Analysis shows that bed height does not affect the initial high level of VFA concentration at all the bed heights used. A similar result was also obtained in the previous section using bed heights of 0.26 and 0.51 metres at a 2:1 solid : liquid ratio. Bed height does however affect the average lower steady concentration of VFA's. If these concentrations are examined for bed heights of 1.02 metres and 0.51 metres, along with those obtained previously for bed heights of 0.51 and 0.26 metres, then it can be seen from Table 4.13 that with increasing bed height there is a concurrent increase in liquor VFA concentration, all experiments reported above taking place under identical operating conditions.

Therefore a unit volume of liquor will be in contact with the bed for longer periods with increasing bed height. Thus substrate within the liquor will be available to proportionally more attached bacteria, allowing for greater conversion rates, and hence biogas production. In addition 'free' bacteria within the liquor will contact a proportionally greater surface of solid matrix, and the acid producing bacteria present will consequently give rise to a higher level of volatile fatty acids as shown in Figure 4.13. This factor will be further discussed in section 4.4.4.5.

Table 4.13 Effect of Bed Height on Liquor VFA Concentration

Bed Height (metres)	0.26	0.51	0.51	1.02
Average liquor VFA Concentration (ppm)	1997	2274	2195	2406
	Substrate - table 4.1		Substrate - table 4.2	

4.4.4.4 Gas Composition

Methane content of the biogas reached its maximum level after 11 days of operation in all cases. Average methane content after this time was, at a bed height of 0.51 metres = 60.27 (±1.31)%, 1.02 metres = 60.19 (±0.92)%, 1.53 metres = 61.08 (±1.27)% and 2.05 metres = 60.84 (±1.81)%. Steady state methane content again seems to reflect steady state volatile fatty acid concentration. Higher VFA concentrations causing a small reduction in methane content, possibly due to minor inhibition of methanogenic bacteria.

4.4.4.5 Adenosine 5'triphosphate Concentration

Analysis of the ATP concentration in both the liquid and solids phases at bed heights of 0.51 and 1.02 metres indicate the level of colonisation to be similar in each case. ATP concentration in the solid phase was 10.51 (±0.51) x 10^{-6} mol/kg at a bed height of 0.51 metres, and 11.65 (±0.72) x 10^{-6} mol/kg at a bed height of 1.02 metres. The ATP concentration in the liquor

being 1.38 (±0.31) x 10^{-6} M and 1.32 x 10^{-6} M respectively. It is unlikely that this small variation was responsible for increased VFA concentration and thus gas production at the greater bed height. It seems likely therefore that increased residence time of the liquor was responsible; acid forming bacteria within the liquor being able to degrade more polymeric substrate, and the attached methanogens converting more acetate to methane and carbon dioxide.

4.4.4.6 Solids Losses

The variation of average solids losses with bed height bear a close relationship to the gas yield obtained. The results are shown in Figure 4.14 and Table 4.14. The solids losses at a bed height of 1.53 metres are clearly greatest. Due to the blocking of the bed, however, which occurred shortly before the end of the digestion period, operation of percolating packed bed digesters at this height is clearly not practicable. The solids destruction obtained from this type of digestion is very substantial, compared with the findings of other workers. For example Goldberg et al (1981) obtained a total solids reduction of 38% using a pig manure and straw substrate of 21% dry matter at a 60 day detention period.

4.4.4.7 Liquor Composition

Liquor pH was maintained at between 7.43 and 7.62 in all cases, and did not drop below 6.98 in the initial stages of digestion, probably due to the high levels of alkalinity recorded,

Table 4.14 Effect of Bed Height on Solids Losses

Bed Height (metres)	0.51	1.02	1.52	2.05
Weight T.S. added (kg)	1.664	3.328	4.991	6.932
Weight V.S. added (kg)	1.201	2.402	3.604	5.005
Weight Cellulose added (kg)	0.893	1.787	2.680	3.723
Total Solids destroyed (%)	34.6	42.1	46.0	31.7
Volatile Solids destroyed (%)	40.5	49.1	49.3	38.8
Cellulose destroyed (%)	58.7	59.3	61.9	55.2
Average Liquor Total Solids Content	2.12 ±0.13	2.32 ±0.20	2.14 ±0.17	2.34 ±0.27

between 6525 and 8250 mg/l of calcium carbonate.

Bed height did not appear to influence the liquor total solids concentration, as might be expected using the same solid : liquid ratio. The average total solids contents being shown in Table 4.14.

Liquor total nitrogen concentration increased by a similar amount at each bed height, on average 953 (±87) ppm. Ammonaical nitrogen increased by a slightly smaller amount, 847 (±62) ppm. There was thus a small increase in non-ammoniacal nitrogen probably caused by an increase in lignin masked proteinaceous material. The loss of total nitrogen from the solid phase was less than had been previously encountered, and is possibly a function of the lower initial nitrogen content. It is feasible that for a given weight of manure-straw substrate a finite amount of nitrogen is permanently attached, the remainder could be dissimilated. Total nitrogen losses from the solid phase averaged 594ppm. Thus 80% of this apparent nitrogen loss was to the liquor phase (at a solid : liquid ratio of 2:1, each unit of nitrogen lost from the bed will be the equivalent of two units in the liquor phase). The remainder of the nitrogen loss may be accounted for by experimental error, ammonia volatalisation or denitrification.

4.4.4.8 General Observations

Bed height in percolating packed bed digesters was shown to be an important factor in influencing gas production. It would appear that this was due to an increase in liquor residence time within the bed, as its height was increased at a constant recirculation rate. This will therefore increase the availability

of metabolites to attached bacteria and bring hydrolytic and fermentative bacteria suspended in the liquor into contact with proportionately more substrate.

Operation of digesters at their maximum bed height will thus increase gas production and solids destruction, and hence improve their economic viability. Operation above a certain bed height leads to blockage caused by compaction of the digesting material. There is thus an optimum bed height, above which blockages are likely to occur and below which gas production will be poorer.

4.5 Conclusions

1) Results obtained in this section confirm previous results in that operation was stable over a wide range of operating conditions.

2) The system behaves as would be expected with temperature, a gas yield of 0.305 m^3/kg VS added being obtained at 35°C while at 25°C the yield was 0.172 m^3/kg VS added. However due to increased heating requirements with an increased temperature of operation, a temperature below 35°C may yield greater quantities of utilisible biogas.

3) Lower temperatures appear to decrease the activity of methanogenic bacteria to a proportionately greater extent than non-methanogenic bacteria as was indicated by higher liquor VFA concentration and lower methane content in the biogas during the initial stages of digestion.

4) Liquor recirculation rates in the range used appear to have little effect on gas yield, though solids degradation was greatest at a rate of 3.0 litres/hr. Though zero recirculation could be used, with considerable savings in energy input, gas production however was found to decrease rapidly in the latter stages of digestion and linking of digesters in series to form a semi-continuous system would not be possible.

5) The solid : liquid ratio was shown to be highly important with a ratio of 2:1 proving to be optimum for gas production and solids loss, showing a 38% increase in gas yield compared to a ratio of 1:1.

6) Increased bed height was found to increase gas yield up to a critical point when compaction followed by blocking occurred. Using a manure-straw mixture with a digester diameter of 0.18 metres this was found to be 1.5 metres. Efficiency of the digestion process was increased with bed height due to increased liquor residence time.

7) It was found that an increased steady state liquor VFA concentration led to an increase in gas production, volatile fatty acids enhancing gas production providing they do not reach inhibitory levels.

8) In agreement with other workers' findings (e.g. Hills, 1979)

gas yield was found to be improved with higher Carbon : Nitrogen ratios, although other variables such as initial ammoniacal nitrogen content may also be important.

9) Gas yields of up to 0.31 m^3/kg VS added together with volatile solids losses of 47.8% were obtained after 40 days of operation, comparable with those of other workers. Methane content of the biogas was found to be in the range 59.1 to 63.5%, within the range expected from straw-manure substrates.

10) The majority of nitrogenous material lost from the solid matrix during digestion was found to be transferred to the liquor phase. The fertiliser value of the waste was thus maintained, providing both solid and liquid phases are used as a fertiliser after digestion.

11) The effect of varying digester diameter was not investigated in this study, it will however influence the superficial velocity of the liquor through the bed. It might also be expected that with greater cross-sectional areas compaction of the packed bed will occur more readily when straw fibres lose their rigidity as digestion progresses.

Figure 4.1 Increase in Gas Yield with Increased Temperature

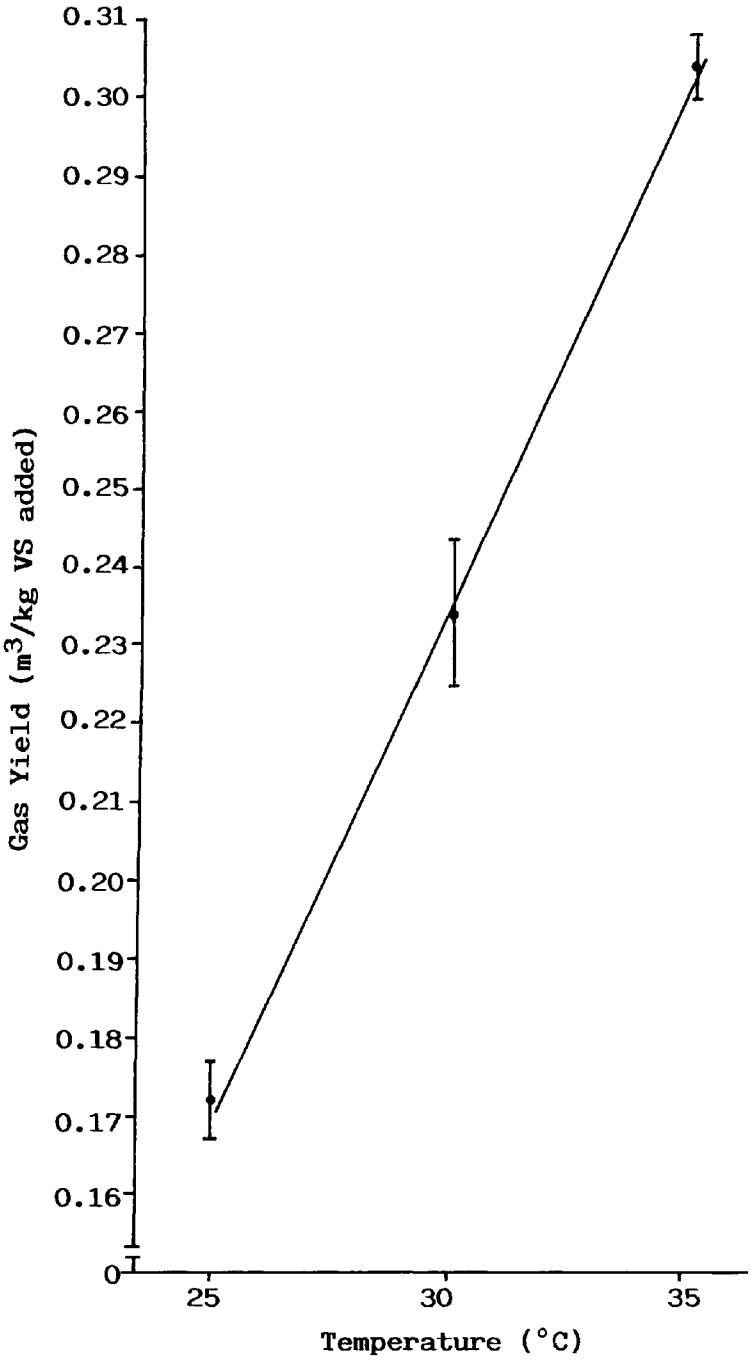


Figure 4.2 Effect of Temperature on Daily Gas Production

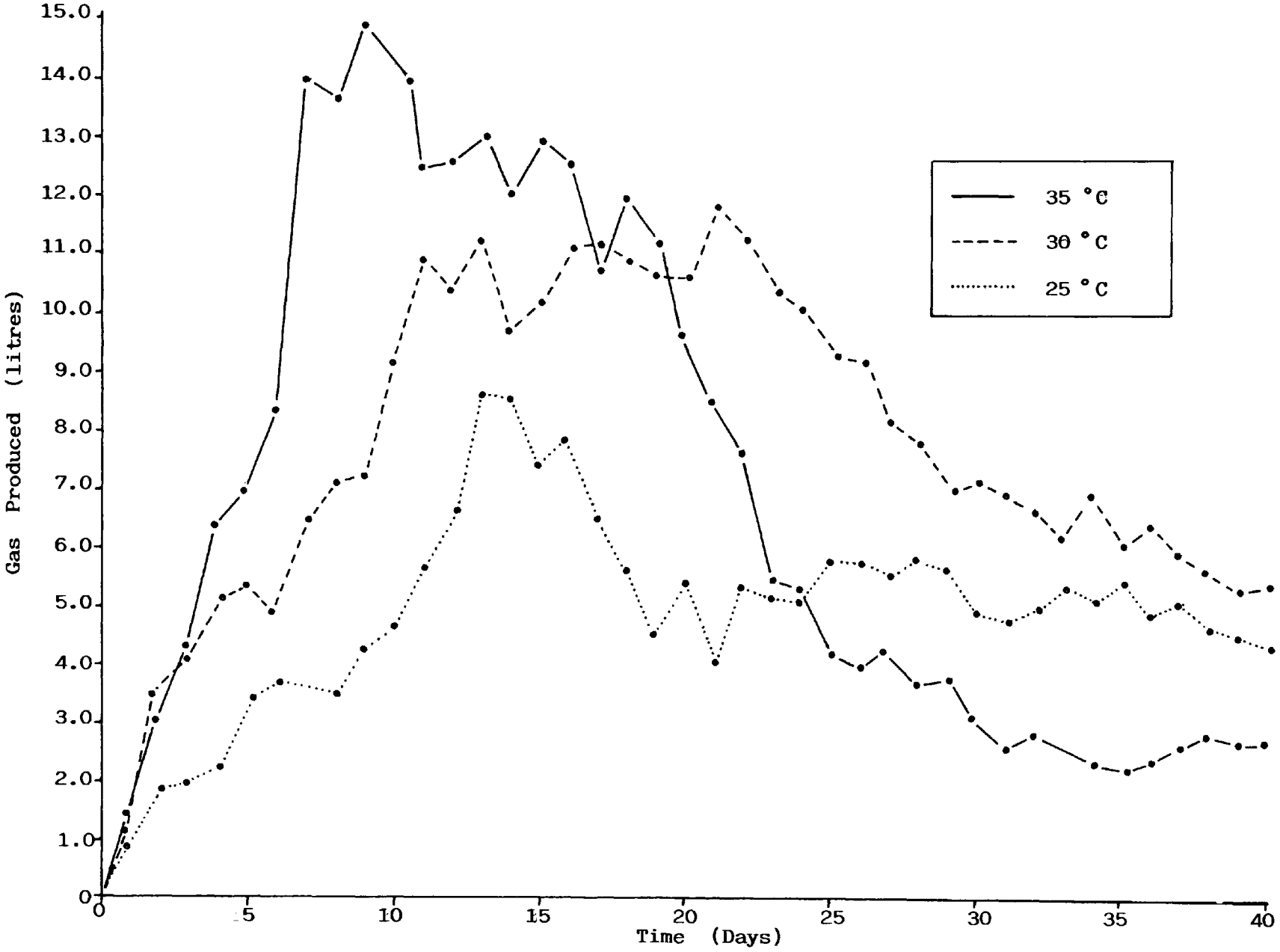


Figure 4.3 Effect of Temperature on Liquor Volatile Fatty Acid Concentration

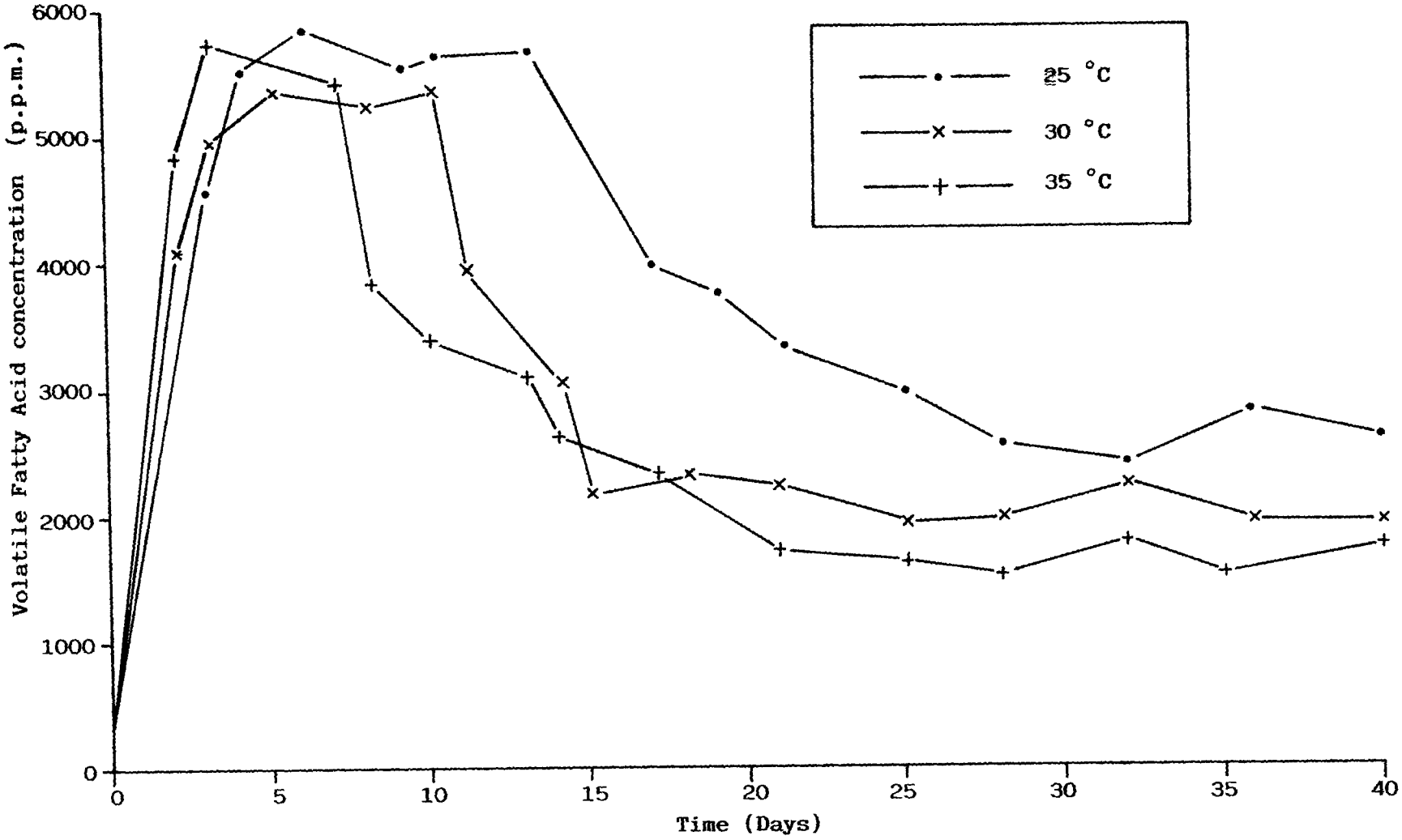


Figure 4.4 Methane Content of Biogas with Increased Temperature

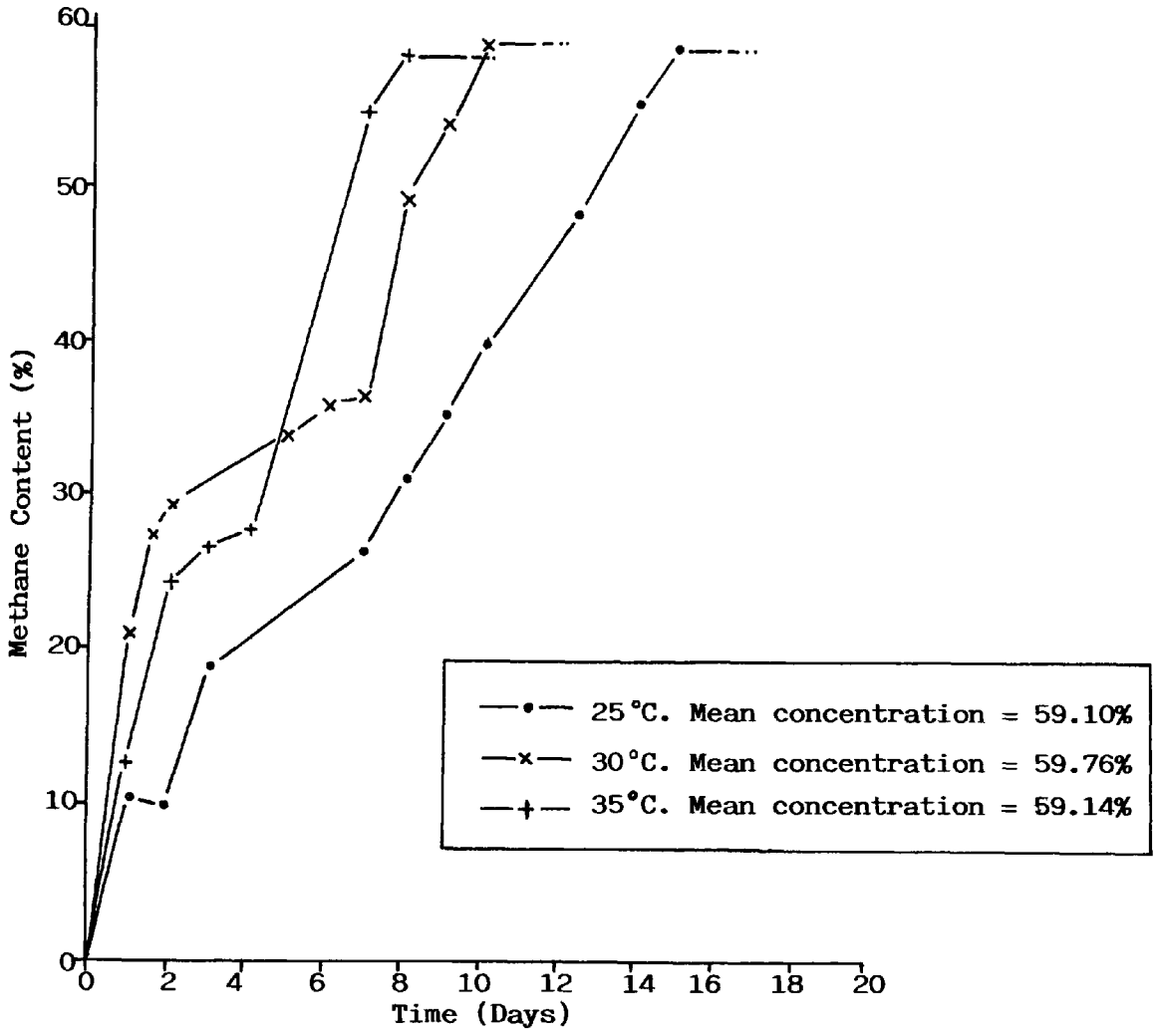


Figure 4.5 Effect of Recirculation Rate on Gas Yield

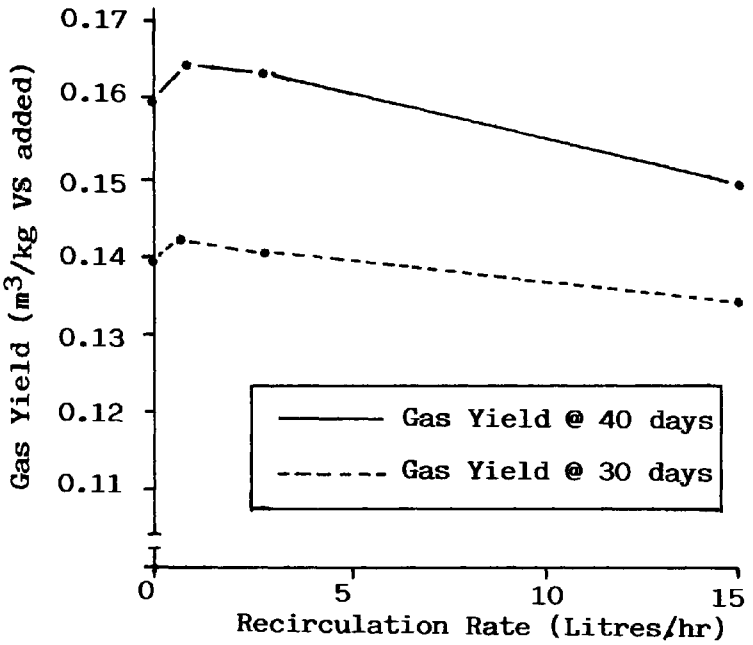


Figure 4.6 Effect of Recirculation Rate on Gas Production

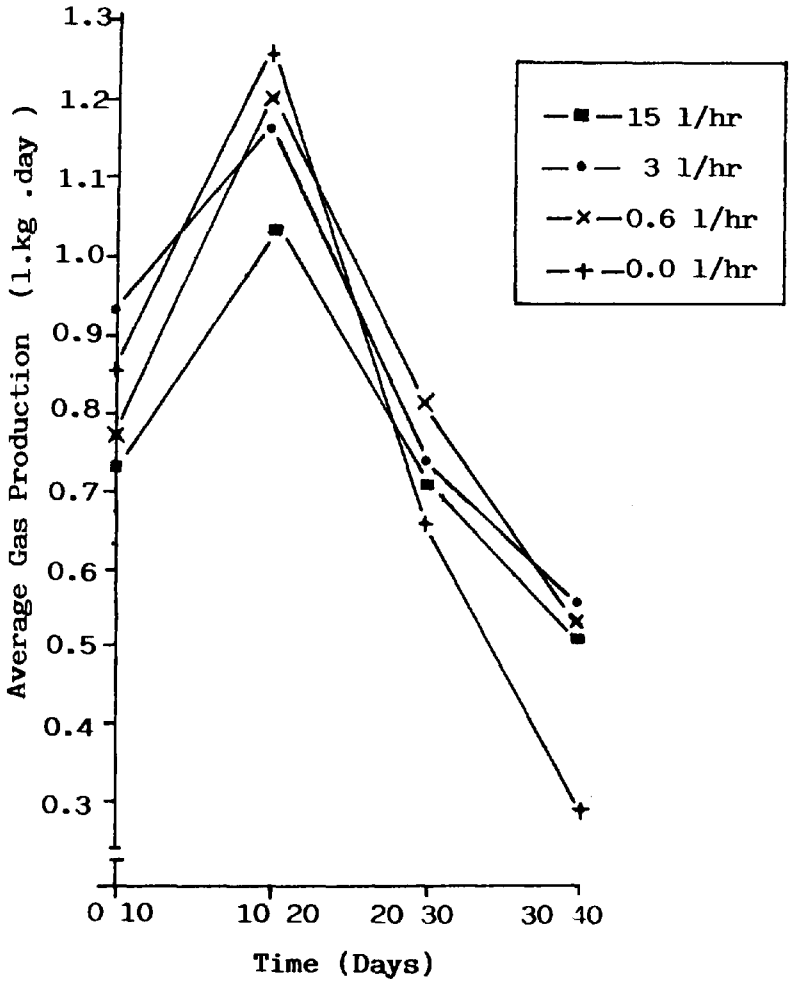


Figure 4.7 Effect of Recirculation Rate on Solids Losses

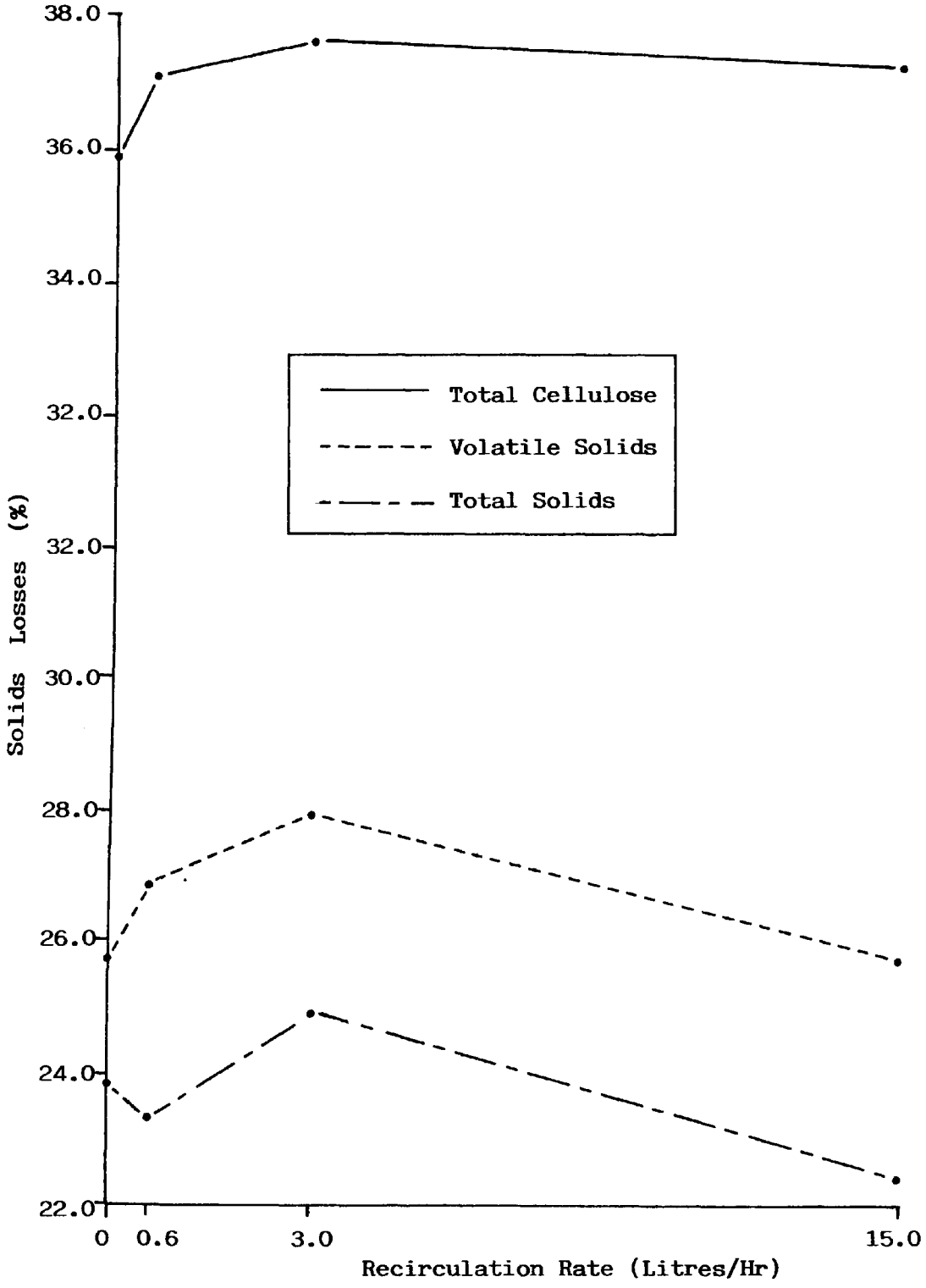


Figure 4.8 Effect of Solid:Liquid Ratio on Gas Yield

(Note: The results shown for a 2:1 ratio are the combined averages for solids loadings of 6kg and 3kg. Bars represent standard deviation.)

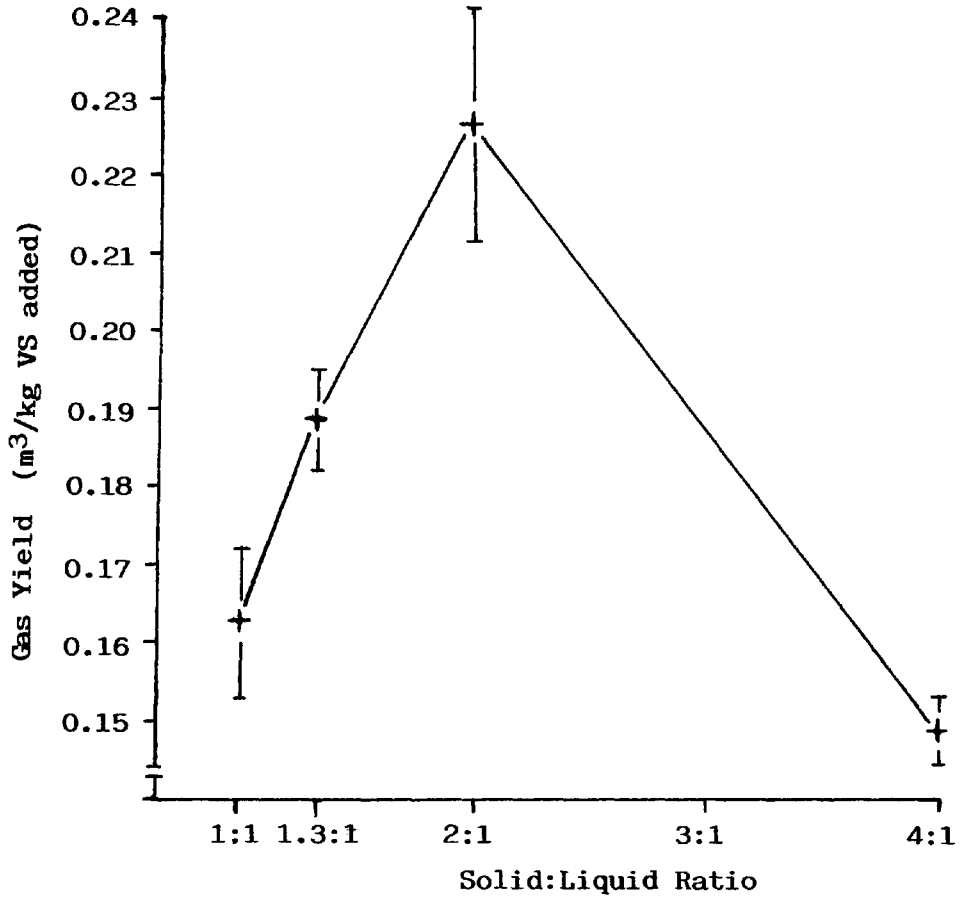


Figure 4.9 Liquor Volatile Fatty Acid Concentration with Increased Solid:Liquid Ratio

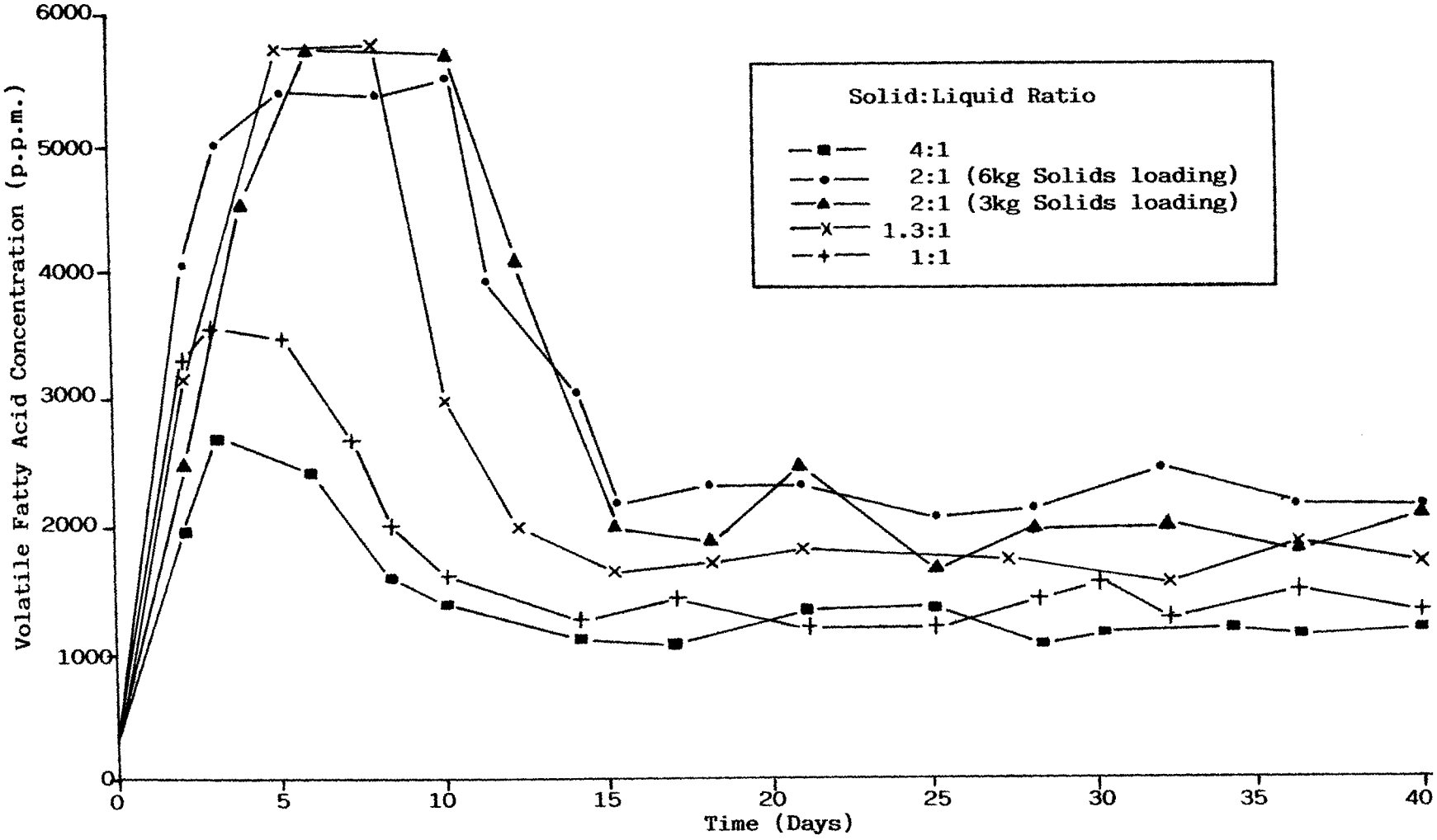


Figure 4.10.a. Effect of Solid:Liquid Ratio on Solids Losses

* See note Figure 4.8.

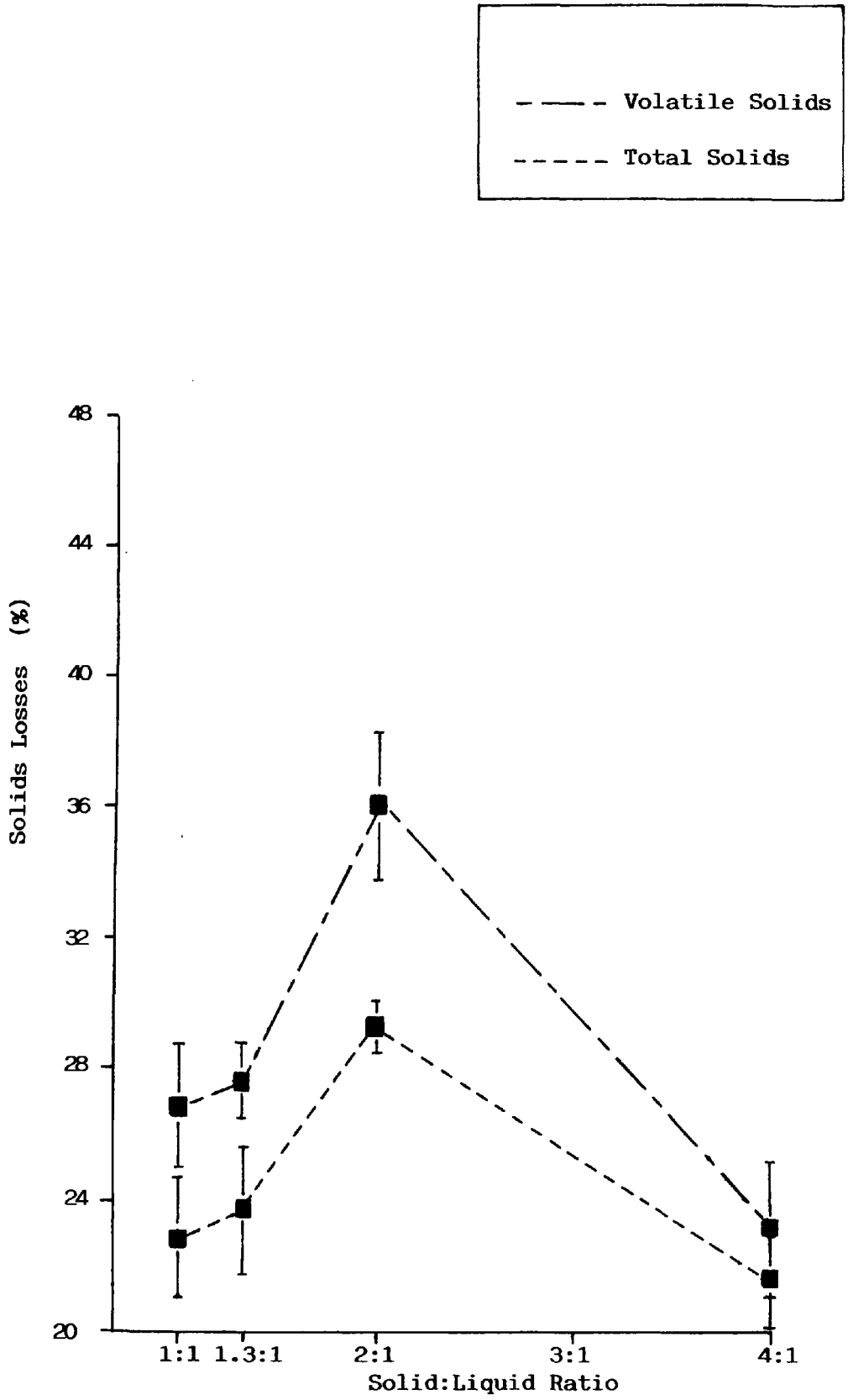


Figure 4.10.b Effect of Solid:Liquid Ratio on Liquor Total Solids Content

(Bar shows standard deviation)

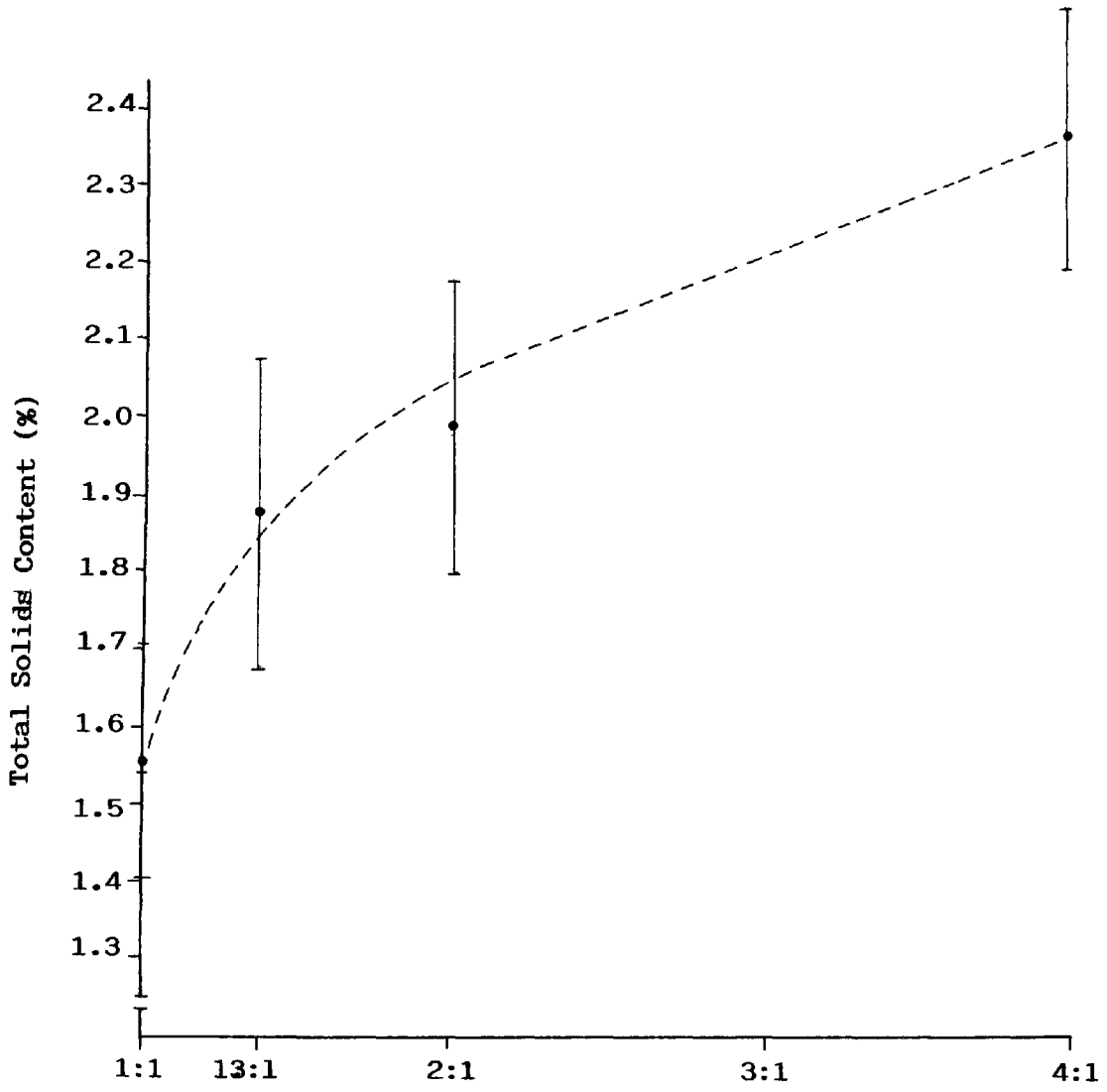


Figure 4.11 Schematic Diagram of Digester Operation at Increased Bed Heights

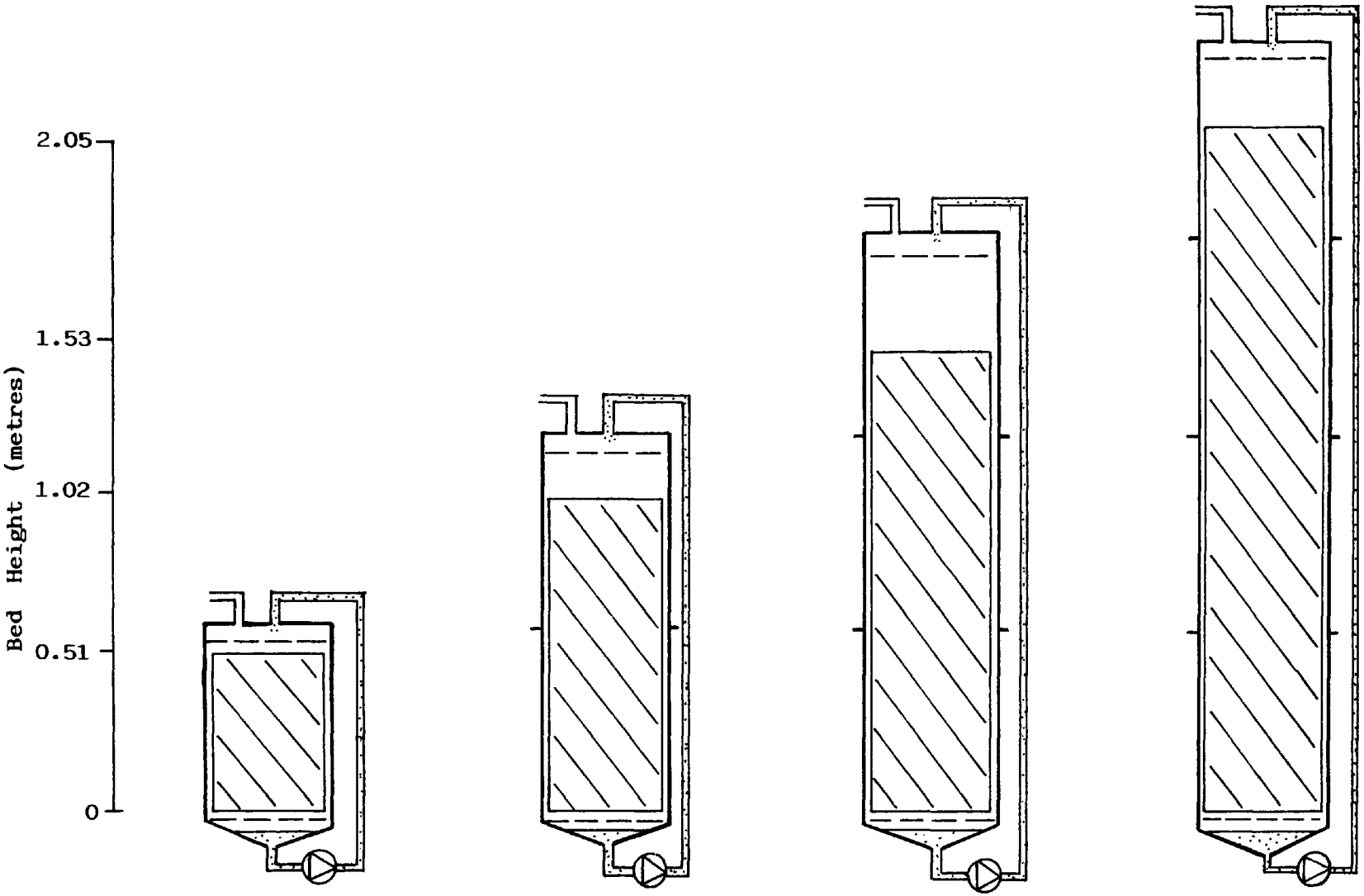


Figure 4.12 Cumulative Gas Production at Increased Bed Heights

(Note; All results are corrected to be equivalent to a 6kg loading)

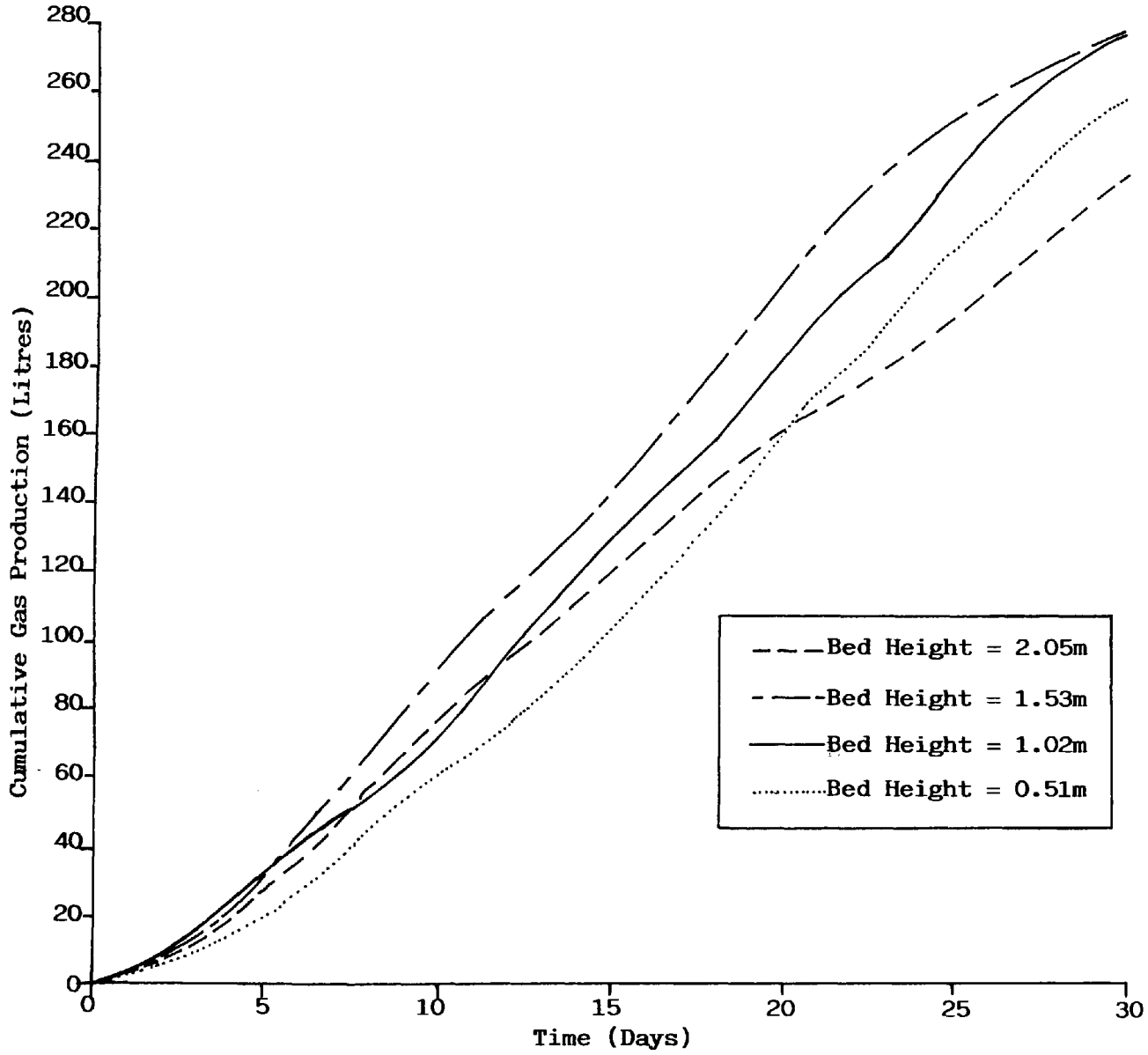


Figure 4.13 Effect of Bed Height on Liquor Volatile Fatty Acid Concentration

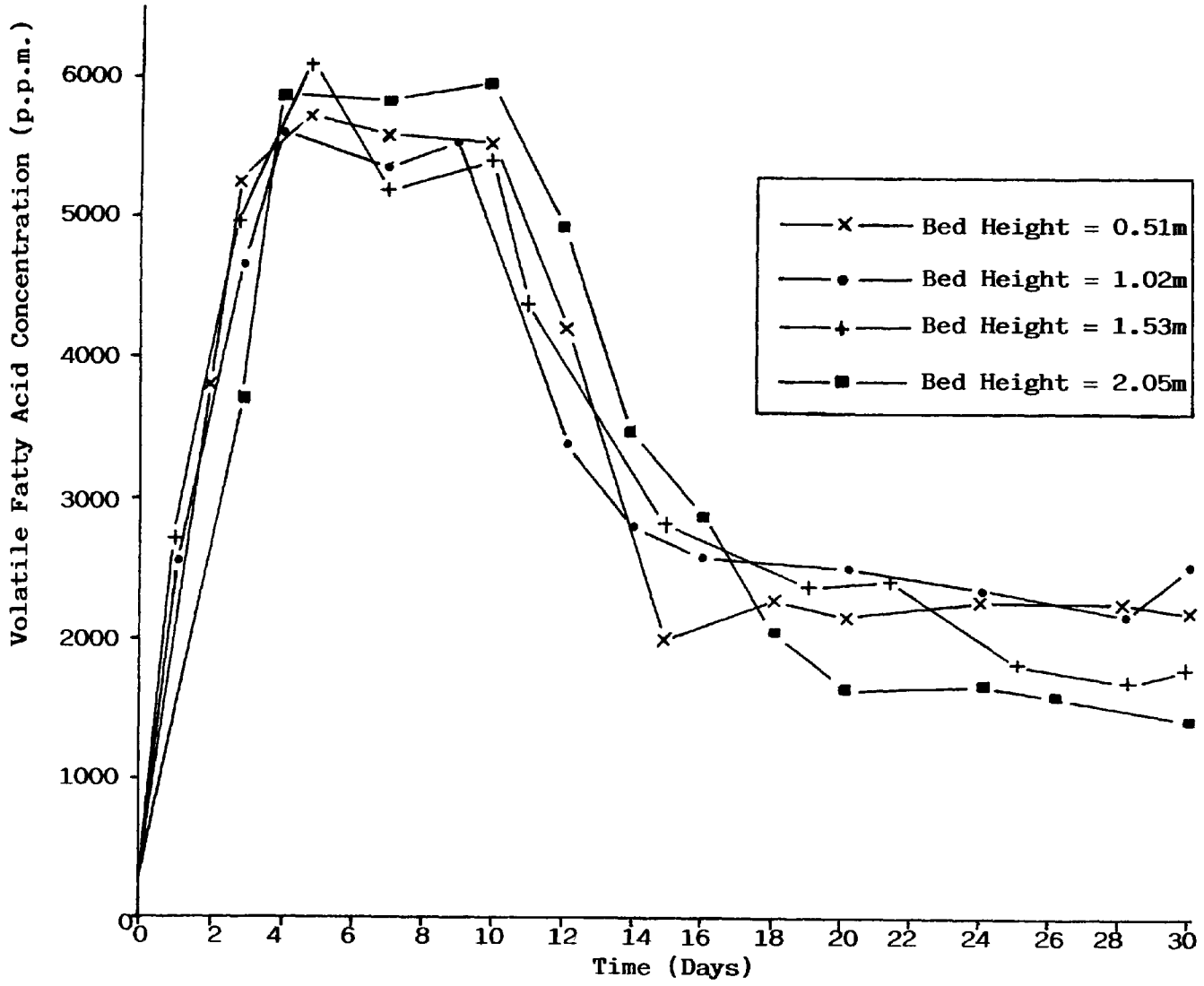


Figure 4.14 Effect of Bed Height on Solids Losses

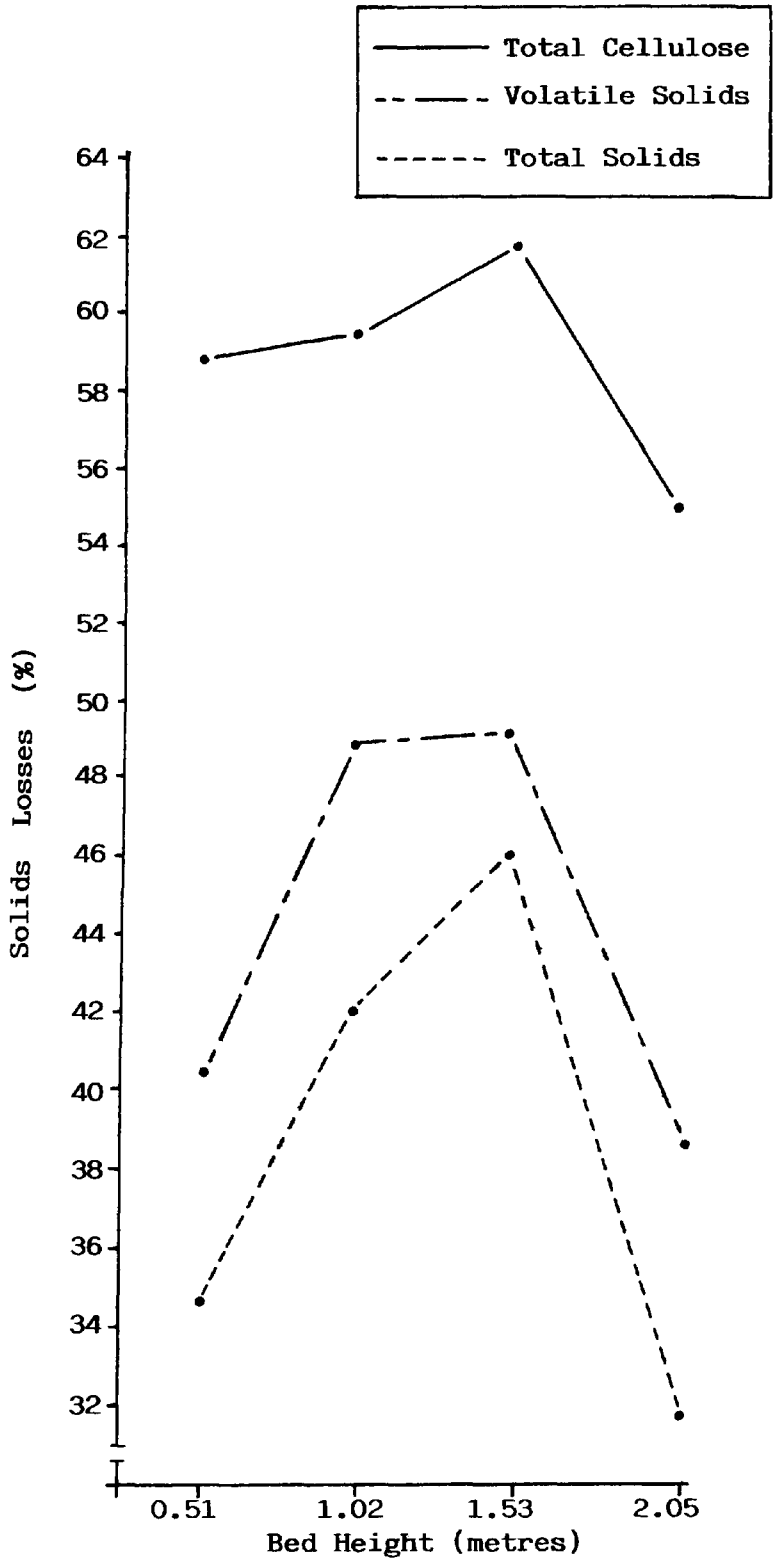
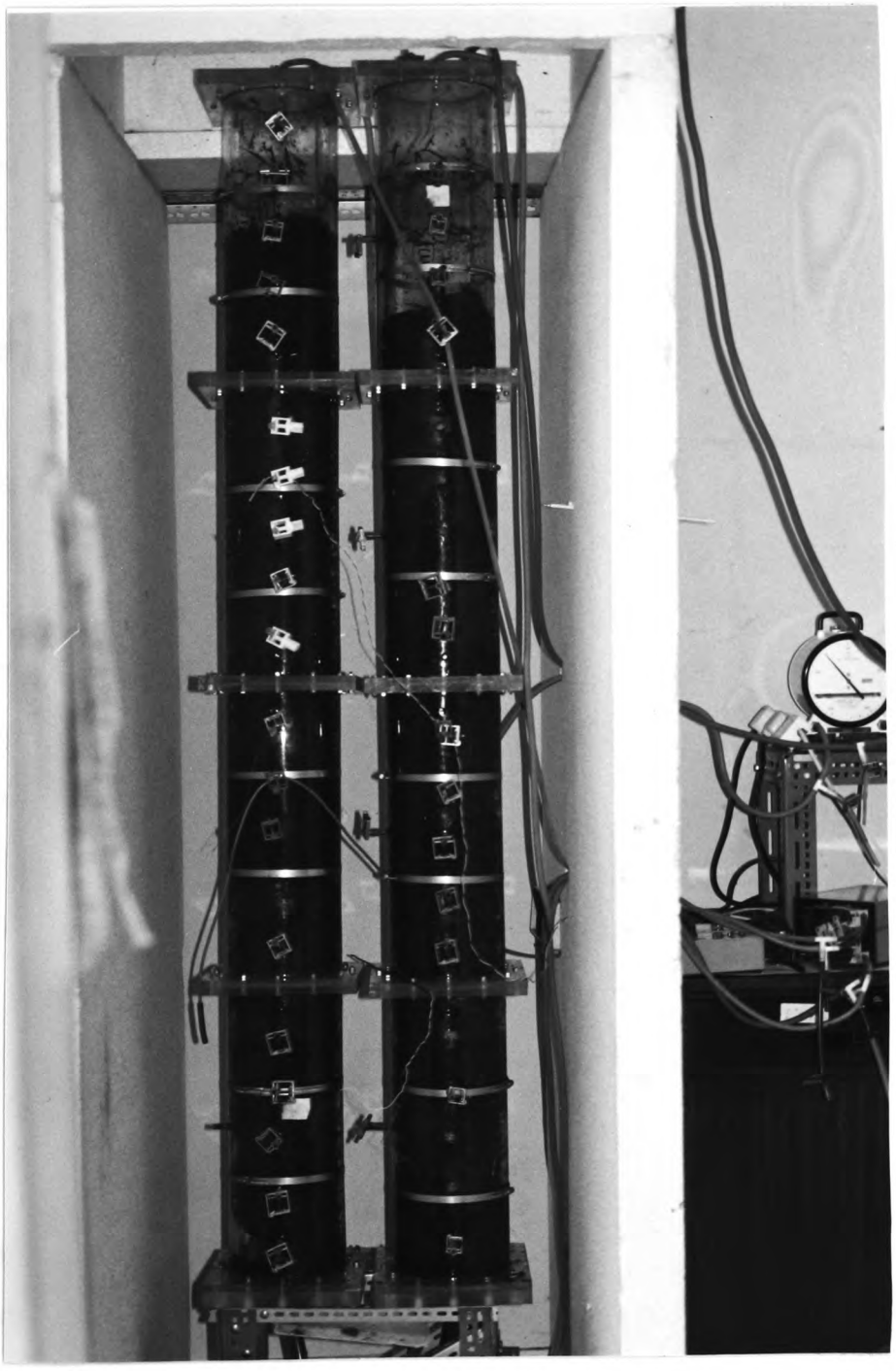


Plate 4.1 Duplicate Operation of Digesters at Increased Bed Height (2.05 metres)



CHAPTER 5

BIOLOGICAL ASSESSMENT OF DIGESTER PERFORMANCE

5.1 Introduction

This chapter concerns itself with the assessment of digesters using parameters such as bacterial enumeration, by indirect estimation of viable numbers, analysis of both the solid and liquid phases using scanning electron microscopy, and the use of Adenosine triphosphate as a guide to bacterial numbers. In addition the relative rates of gas production (and methane production) from the solid and liquid phases was also monitored. Hydrogen sulphide concentration within the biogas was closely monitored in order to give an insight to the populations of sulphate reducing bacteria within the system. In addition, the particle size distribution within the liquor phase was analysed to determine the physical stability of the system relating to blockages of the recirculation system, and also to validate the use of ATP analysis for bacterial populations.

These assessments will lead to a greater understanding of digester performance and may lead to improved modes of digester operation which could improve the economics of the process.

5.2.1 Digester Operation

In this series of experiments eight digesters were operated (two identical sets of four). The digesters were operated in a batch mode with a solid : liquid ratio of 2 : 1 (using 6kg of solid substrate and 3 litres of liquor) corresponding to a bed height of 0.51 metres, with a recirculation rate of 3 litres.hr^{-1} at 30°C. Analysis was conducted on the solid and liquid phases before start up (time = 0), digestion was then commenced. Two digesters were then dismantled and the relevant analyses conducted after 7, 14, 21 and 28 days of operation. These times were chosen a) to allow a convenient and rational approach to digester monitoring, and b) so digestion was examined during the unstable start-up phase and through into the stable phase, the stable phase being that when parameters such as volatile fatty acid concentrations are at low and steady levels, which has been shown to be after around 15 days of operation (Chapter 3).

5.2.2 Digester Monitoring

The digesters were monitored after each stage (ie after 7, 14, 21 and 28 days) for their solids composition in order to determine solids losses. The liquor was monitored twice weekly from each digester for pH, volatile fatty acid concentration, total solids, volatile solids and weekly for total nitrogen and ammoniacal nitrogen as previously described. These analyses were conducted in order to ensure that digestion was proceding normally, for example that liquor volatile fatty acids and ammoniacal

nitrogen concentration followed patterns previously obtained (see Chapters 3 and 4). Since this was found to be so, these aspects of the digester operation are not discussed in this section. Gas production was also monitored daily again to ensure that digestion was proceding normally, and also for the determination of the relative rates of gas production from the solid and liquid phases. Gas composition was monitored daily for methane content in order to determine the relative rates of methane production at each stage of digestion.

In addition the hydrogen sulphide concentrations of the biogas along with liquor ATP concentrations were closely followed throughout the course of digestion.

After each digestion period, the particle size distribution of solids entrained in the liquor were determined by sieving, and the solids losses estimated, as were the ATP levels within the solid phase. In addition after each digestion period the bacterial population of both the solid and liquid phases was analysed by scanning electron microscopy, and the relative proportion of gas produced from the solid and liquid phases estimated.

After 21 days of operation, when digestion was stable, indicated by the steady liquor volatile fatty acid concentration, the populations of both methanogenic and non-methanogenic bacteria were estimated by the methods previously described in Chapter 2.

5.2.3 Substrate Composition

The solid substrate was analysed from eight representative samples for its proximate constituents and is shown in table 5.1. It can be seen that the substrate is similar to those used in the preceding Chapters and it could therefore be reasonably assumed that digestion would proceed as previously described, and this was indeed found to be so.

5.3 Results and Discussion

5.3.1 Liquor Particle Size Distribution

Particle size distribution was determined as previously described by passing 1 litre of liquor through the mesh of three sieves of sizes 1000μm, 250μm and 75μm. The individual fractions were collected and dried and the percentage size distribution calculated. These analyses were conducted on the inoculum and on the digester liquor after 7, 14, 21 and 28 days of operation. The results obtained are shown in Table 5.2 and Figure 5.1. It can be seen that particles of sizes greater than 1000μm rapidly diminished, being reduced by 81% in 7 days. Similarly particles of sizes 250μm - 1000μm and 75μm - 250μm are reduced by 88% and 57% respectively over the same time period. At the same time particles of less than 75μm increased by some 34% over the first 7 day period. During the rest of the digestion these trends in particle size distribution were continued, but the changes are much less dramatic, the majority of change occuring in the first seven days

of operation. There is thus a clear trend in particle size reduction, while the overall dry matter content of the liquor remains approximately constant at 2.13 (+ 0.21%). The bacterial population of the liquor will be accounted for in the less than 75μm fraction except those attached to the surfaces of larger

Table 5.1 Substrate Composition

	mean	St.dev.
Total Solids (%)	21.8	0.73
Volatile Solids (% of T.S.)	79.65	2.05
Volatile Fatty Acids (ppm)	2071	426
Total Nitrogen (ppm)	5644	395
Ammoniacal Nitrogen (ppm)	1763	284
Cellulose (% of wet weight)	11.91	0.37
Adenosine triphosphate ($mol.kg^{-1} \times 10^{-6}$)	0.70	0.21
Carbon : Nitrogen ration	18.3 : 1	-

Table 5.2 Liquor Particle Size Distribution

Time of Analysis	Sieve Size	Particle Size	Percentage Dry Wt. Retained
0 days	1000μm	>1000μm	6.44
	250μm	250-1000μm	15.96
	75μm	75-250μm	6.00
	pan	<75μm	71.51
		Total	99.91
7 days	1000μm	>1000μm	1.22
	250μm	250-1000μm	1.77
	75μm	75-250μm	2.53
	pan	>75μm	94.68
		Total	100.20
14 days	1000μm	>1000μm	1.40
	250μm	250-1000μm	1.68
	75μm	75-250μm	1.68
	pan	<75μm	95.25
		Total	100.01
21 days	1000μm	>1000μm	0.00
	250μm	250-1000μm	0.38
	75μm	75-250μm	0.36
	pan	<75μm	99.22
		Total	99.96
28 days	1000μm	>1000μm	0.22
	250μm	250-1000μm	0.47
	75μm	75-250μm	1.01
	pan	<75μm	98.36
		Total	100.06

particles.

The rapidity of the change in particle size distribution indicated that it was not due to bacterial action alone (the digestion of particles causing a reduction in their size), but also a physical filtration effect. As the liquor and the particles it contained were recirculated, larger ones were trapped in the interstices of the solid matrix, and were thus filtered from the liquor. These results confirm those obtained and presented in Chapter 3 where it was found that the cellulose concentration in the liquor decreased by 95% in the course of a 40 day digestion.

Thus from the average dry matter content of the liquor of 2.13% less than 2% of this was composed of particles coarser than 75μm. This is important as there was no build-up of particles to block the recirculation line. Blockages of the bed due to these particles is unlikely due to the numerous passageways through which liquor can flow through the bed. This will be of great importance in a full scale process, as blockages may lead to down-time for the digester and could adversely affect its gas production and hence economic viability.

The low levels of particulate solids may also have important implications in the determination of Adenosine triphosphate in the liquor phase. The extraction of ATP could be hindered by the bacteria being attached to solid particles, in positions where they are inaccessible to the sulphuric acid. Thus with low levels of particulate solids, fewer bacteria will be attached and thus the ATP concentration obtained will be more accurate.

5.3.2 Hydrogen Sulphide Content of the Biogas

The Hydrogen sulphide content of the biogas was monitored twice-weekly by the method previously described, and the results obtained are shown in Figure 5.2.

It can be seen that the hydrogen sulphide concentration increased to very high levels during the early stages of digestion. It then rapidly decreased, and after around 16 days reached a low level of approximately 250ppm where it remained throughout the remainder of the experiment. There are a number of explanations relating to this trend in hydrogen sulphide concentration which was present in all experiments conducted.

Stetter and Gaag (1983) have reported that methanogenic bacteria form large amounts of hydrogen sulphide in the presence of molecular sulphur, in addition to the production of methane. It has been suggested that by this means, methanogens can create their own anaerobic environment. In the orders Methanococcales and Methanobacteriales the production of hydrogen sulphide causes a lowering of methane production. It has also been shown that high hydrogen sulphide concentrations lead to a growth inhibition of methanogens, and in its toxic undissociated form approximately $60mg.l^{-1}$ free hydrogen sulphide leads to a 50% inhibition of methanogenesis (Kroiss and Wabnegg, 1983). Sulphate is thus a potent inhibitor of methanogenesis by diverting electrons from methanogenesis. However high sulphate feeds leading to high sulphide concentrations, can be tolerated due to the precipitation of sulphide in insoluble metal complexes (Archer, 1983).

Hydrogen sulphide formation is favoured by most methanogens

in the presence of sulphur, but in all cases some methane is formed. In addition sulphate reducers present such as Desulphovibrio outcompete methanogens when sulphate is not limiting, having greater affinities for hydrogen and acetate (Robinson and Tiedje, 1984). Methanogens compete with sulphate reducers for hydrogen at high sulphate levels which favours the sulphate reducers, however in media with low sulphate concentrations the sulphate reducers will degrade lactate and ethanol provided methanogens are present to remove the hydrogen which is produced.

These therefore could well be contributory factors causing the low levels of methane production when hydrogen sulphide is high:-

1) Methanogens are preferentially producing hydrogen sulphide.
2) Sulphate reducers present are out-competing the methanogens.
3) High hydrogen sulphide levels are inhibiting the production of methane.
4) A combination of the above.

It is clear therefore that a highly degradable sulphur containing compound was present in the substrate, which was readily converted to hydrogen sulphide gas which was removed from the system, and may contribute to the low methane production during the initial stages of digestion. Once this unidentified compound has been degraded hydrogen sulphide in the biogas falls to very low levels, and was present possibly as a result of the degradation of sulphur containing amino acids such as cysteine.

An interesting phenomenon was noted during the operation of digesters in a semi-continuous mode. When a fresh digester was

added, the liquor passing from it to the established digester caused the initial digester to show an increased rate of gas production (see Chapters 6 and 7). Also increased was the hydrogen sulphide content of the biogas to around 2000ppm. There was however no decrease in the methane content of the biogas. The highly degradable sulphur component is therefore soluble as it is transported via the recirculation stream. As the methane content of the biogas is unaffected it may reasonably be concluded that it is a product of the sulphate reducers present, the methanogens being unable to compete for the sulphur containing compound.

5.3.3 Relative Rates of Gas Production from the Solid and Liquid Phases

The volume of biogas and methane produced by each phase was determined after 0, 7, 14, 21 and 28 days of operation by the method previously described (Section 2.4.12). In this manner it was possible to examine the contributions of the solid and liquid phases to the total biogas production, allowing an insight into the levels of biomass present in each phase. In the following results it was assumed that before inoculation the solid phase was producing no biogas. It is probable however that some gas (which will be almost totally carbon dioxide) was produced, but the volume is unknown and likely to be very small.

It can be seen from the results that biogas production from the digesters was closely similar to that previously obtained (Chapter 4) under the same operating conditions (Fig 5.3a and Table 5.3). Initially (time = 0) only the inoculum was producing biogas

of 64% methane, however 7 days after start up the solid phase accounted for 85% of the biogas produced (Fig. 5.3b) but with the biogas from the liquor phase having a greater percentage of methane. After 14 days of operation 96% of the biogas originated from the solid phase, with the biogas from the liquor phase having a lower methane content. This trend then continued with the solid phase accounting for over 98% of the biogas and the liquor phase continuing to produce less methane (Fig.5.3c)

The inoculation of the bed was thus very rapid, and after only 10 days, 90% of the biogas was produced from within the bed. It must be said, however, that liquor entrapped within the solid matrix will be contributing to this total. It can be seen that in the early stages of digestion the methane content of the biogas from the liquor phase was higher than that from the solid phase. During the course of digestion however the methane content of the liquor biogas decreases to well below that of the solid phase.

The results thus indicate that colonisation of the solid matrix occurs very rapidly in the early stages of digestion. The more gradual drop in the methane content of the 'liquor biogas' tends to suggest however that colonisation of the bed by the methanogenic bacteria occurs at a slower rate than the colonisation by the whole microbial population. This may indicate that methanogenic bacteria find it more difficult to attach to the bed before the straw fibres have been attacked and 'roughened' up by the exo-cellular cellulases. Huysman et al (1983) have shown that surface roughness is one of the most important factors governing fixed film colonisation.

This rapid attachment could be of considerable importance,

as Parkin and Speece (1983) have shown that attached bacteria are better able to respond to an influx of toxic compounds.

The results presented here bear out those previously obtained which indicated a rapid colonisation of the solid matrix, and have shown that both methanogenic and non-methanogenic bacteria are able to do so, with the attachment of methanogens being slightly slower. This may be a result of volatile fatty acid inhibition and hydrogen sulphide inhibition causing a slowing of methanogenic growth rate, and hence though these bacteria attach themselves readily their growth is inhibited leading to a depression of methane production from within the solid matrix. This theory is indeed borne out by the rapid attainment of high methane levels from digesters operated in a semi-continuous mode (Section 6.4.2), where high levels of inhibitory volatile fatty acids were not evident.

5.3.4 Adenosine 5' triphosphate levels in the Solid and Liquid Phases

The microbial biomass of digesters can be estimated in several ways, including the measurement of microbial nucleic acids and amino acids, bacterial diaminopimelic acid and isotope incorporation techniques (Stern and Hoover, 1979). These methods generally however measure total biomass i.e. live cells plus a proportion of dead cells. Direct cell counting can be used, but part of the biomass present will be attached to particulate matter and therefore difficult to measure by these techniques. Two more recent methods are the measurement of coenzyme F_{420} by fluorimetric

monitoring (Binot et al, 1981), F_{420} being present only in methanogenic bacteria this method therefore does not take into account non-methanogenic bacteria present. The second is the measurement of adenosine 5' triphosphate which gives an indication of only live biomass, or at least its metabolic activity (Wolstrup and Jensen, 1976). The method used was after that of Wallace and West (1982), and though it cannot at present be directly related to bacterial numbers due to different ATP pool sizes and metabolic rates, it does give an insight into changes in population size during the course of digestion.

The results of ATP concentrations in the liquid and solid phases are shown in Table 5.4 and Figure 5.4. It can be seen that the initially high level of ATP in the liquor phase decreased rapidly to a base level where it remained throughout the remainder of the operational period. The decrease was presumably due to attachment and retention of biomass within the bed. This indeed appears to be the case, as ATP levels within the solid phase rapidly increased and appeared to reach a finite maximum level of approximately 10×10^{-6} Mol.Kg^{-1}. Similar results were obtained by Shapiro and Switzenbaum (1984) who found a rapid (almost linear) increase in the biofilm up to between 15 and 20 days of operation, followed by a levelling off period with steady biofilm levels. This is possibly a function of available attachment sites within the bed, which was also indicated by the results of Section 7.3.8. These results concur with those previously obtained which indicated a rapid colonisation of the bed, in particular the rate of gas production and solids degradation.

Table 5.3 Proportional Biogas Production of the Solid and Liquid Phases

Time (days)	0	7	14	21	28
Total Daily Biogas Production (litres)	1.07	6.53	11.25	9.57	6.17
Methane Content (%)	64.3	41.5	59.9	60.2	59.7
Biogas Produced from Liquor Phase (% of total)	100	14.5	6.1	1.3	1.9
Methane Content of Liquor Biogas (%)	64.3	58.6	42.9	39.1	34.6
Biogas from Solid Phase (% of total)	0	85.5	95.9	98.7	98.1

Table 5.4 Adenosine 5' triphosphate Concentration in the Solid and Liquor Phases

Time (days)	Average Liquor Phase ATP Concentration ($\underline{M} \times 10^{-6}$)	Average Solid Phase ATP Concentration ($Mol.kg^{-1} \times 10^{-6}$)
0	3.64 (±0.26)	0.70 (±0.32)
7	1.36 (±0.17)	6.52 (±0.66)
14	1.42 (±0.21)	8.41 (±0.51)
21	1.16 (±0.30)	10.61 (±0.43)
28	1.59 (±0.24)	10.53 (±0.67)

The populations of anaerobic protozoa were not examined in these experiments, and therefore their contribution to the ATP levels obtained is not known. Little literature appears to be available on protozoan populations within digesters, it may therefore be reasonably assumed that the population is small and in percolating packed bed digesters relatively constant.

Thus after approximately 20 days of digestion a dynamic bacterial population has been developed, with steady solid and liquid phase populations. The rate of attachment to, and detachment from the solid matrix, and the growth and decay rate of bacteria being roughly equal, leading to steady population levels.

5.3.5 Microscopic Analysis of the Bacteria Populations

Analysis of the solid matrix and the liquor using Scanning electron microscopy was conducted initially and after 7, 14, 21 and 28 days of operation. The purpose of these experiments being to observe visually the development of biomass, and to allow tentative identification of some bacteria present.

5.3.5.1 Development of Biomass

Initially the biomass load on the solid matrix was low (Plate 5.1a) with the straw fibres being relatively smooth indicating that little cellulolytic activity had taken place prior to digestion. The inoculum however showed a high load of bacteria and numerous types (Plate 5.1b) of great morphological variation. These levels of bacteria were indicated in the determination of

Adenosine 5' triphosphate previously described. After 7 days of operation colonisation of the solid matrix had begun (Plate 5.2a) with many of the bacteria present being cellulolytic as can be seen by the surface damage to the straw fibres. Non-cellulolytic bacteria are also present, particularly within natural or induced deformations of the straw surfaces, roughness of the surface being one of the critical factors governing bacterial attachment (Huysman et al, 1983), together with its wettability, composition and porosity (Oakley et al, 1985). Liquor biomass was now at a reduced level (as was indicated by ATP concentration) though morphological variation is still great with bacteria ranging in size from 0.4 to 1.2μm (Plate 5.2b). After 14 days of operation further degradation of the solid matrix was evident (Plate 5.3a) and large colonies (not shown) of apparently non-cellulolytic bacteria have developed. In certain areas holes through the straw fibres can be seen which are possibly as a result of exocellular cellulases being released from attached bacteria. Analysis of the liquor (Plate 5.3b) again showed large numbers of morphologically diverse bacteria. Many are apparently present as aggregates, though it is possible that this was merely as a result of the filtration and fixing procedure used in preparing the samples.

After 21 days of operation the attachment and cellulolytic action of bacteria can most clearly be seen (Plate 5.4a), though some areas of the straw fibres were as yet unaffected by cellulolytic activity. Plate 5.4b clearly shows the action of exocellular cellulases around some attached bacteria which are forming into long chains as they reproduce across the surface of the straw. In Plate 5.4c the attachment of non-cellulolytic bacteria (possibly

methanogens) is shown. It was very noticeable how their attachment takes place on the roughened areas of the straw fibres. After 28 days the highly irregular surface of the straw caused by the action of cellulases can now be seen (Plate 5.5a). Ridges running through the straw are clearly visible and may be as a result of lignaceous material or variation in the cellulose composition. The liquor (Plate 5.5b) showed a characteristically large and diverse bacterial population.

These analyses have shown that the microbial film develops rapidly, a finding also shown by Schrass and Jewell (1984). Conversely Harvey et al (1984) found that an electron dense mineral deposit was formed around the bacteria, and that few bacteria were tightly attached to the support medium most being entrapped within it. This was not found in this system, and is possibly a function of the biodegradable nature of the support matrix. Analysis of the solid matrix for cellulose content carried out in conjunction with these experiments showed it to be degraded throughout the course of the experiments, and continued when the microbial population had reached its maximum level (Fig.5.5)

5.3.5.2 Tentative Identification of Some Bacteria Present

Although the methods used for the preparation of bacteria for electron microscopy which did not involve critical point drying were not satisfactory, however Plate 2.2 (Chapter 2, Section 2.4.11) an air dried sample, clearly shows a Methanosarcina-like clump of bacteria in the liquor phase (see Zehnder, 1978). Plate 5.1b shows what is possibly a large sarcina-like aggregate similar

to those found by Harvey et al (1984), a variety of other rods and cocci of varying size are also evident. The bacteria arranged within the cleft of the straw fibre in Plate 5.2a appear very similar to the Methanococcii described by Balch et al (1977), though they could equally be non-methanogenic bacteria. This was indeed true of the majority of observations made in this section.

The large (~3μm) cocciod bacterium in Plate 5.3b is possibly Methanococcus vanniellii which range in size between 0.4 and 4.0μm (Leeper, 1982). Of the other bacteria present both rods and spheres many appear to be associated into aggregates, similar to those shown by Robinson (1984).

Plate 5.4a shows numerous bacterial types, both cellulolytic and non-cellulolytic, most of which are not readily identifiable. The presence of bacteria morphologically similar Methanosarcina and Methanospirillium is however noticeable, apparently within deformations in the straw caused by the action of cellulolytic bacteria. Plate 5.4b clearly shows the action of exo-cellular cellulase, from an unidentified short rod shaped cellulolytic bacteria. Bacteria within the pit (centre) may be cellulolytic or simply attached there. If the latter is the case they are possibly Methanobacterium sp. strain AZ (Zehnder, 1978) which show this characteristic morphology, eventually forming rosette-like groups. The aggregate of curved rod shaped bacteria shown in Plate 5.4c are similar in appearance to Methanobrevibacter described by Balch et al (1979). Plate 5.5b shows a wide range of morphologically varied bacteria, and also what is possibly a budding Yeast-like organism.

These investigations have thus shown a wide range of bacterial types both in the liquid and solid phases. It has also been shown in agreement with other workers' findings (Oakely et al, 1985) that bacteria attach more rapidly to uneven surfaces of the support matrix. Also indicated (results not shown) was that many bacteria within the solid matrix are entrapped rather than attached, although attachment would appear to be prevalent.

5.3.6 Enumeration of Methanogenic and Non-methanogenic Bacteria in the Liquor Phase

Bacteria in the liquor phase were enumerated after 21 days of digester operation using the methods previously described, both the investigations presented were conducted in duplicate for two digesters and the results presented are averages.

The results for the estimation of methanogenic bacteria (by headspace gas analysis) are shown in Table 5.5 and Figure 5.6, and those for non-methanogenic bacteria in Table 5.6 and Figure 5.7. The results indicated methanogens to be present at a level of between 10^6 and 10^7 bacteria.ml^{-1} and non-methanogens at a level of between 10^7 and 10^8 bacteria.ml^{-1}.

These results may be lower than the actual value, as no attempt was made to remove all traces of oxygen in the commercially supplied 'oxygen-free' nitrogen. However the resazurin indicator used in both media, indicated a reduced environment. It was assumed that the facultative anaerobes in the liquor would rapidly consume any oxygen present. In addition the figures obtained are in close agreement with those of other workers. Mah and Sussman

Table 5.6 Results of Headspace Gas Analysis used in the Enumeration of Methanogenic Bacteria

Dilution Factor	Average Methane Content	Average Carbon Dioxide Content
10^{-1}	24.46%	5.70%
10^{-2}	17.16%	4.81%
10^{-3}	12.85%	5.00%
10^{-4}	3.85%	2.99%
10^{-5}	2.29%	3.19%
10^{-6}	7.76%	2.36%
10^{-7}	0.16%	2.58%
10^{-8}	0.14%	1.24%
Blank	0.00%	0.02%

Table 5.7 Results of Spectrophotometric Analysis used in the Enumeration of Non-Methanogenic Bacteria

Dilution Factor	Average OD_{600}
10^{-1}	0.61
10^{-2}	0.50
10^{-3}	0.57
10^{-4}	0.41
10^{-5}	0.32
10^{-6}	0.29
10^{-7}	0.10
10^{-8}	0.06
10^{-9}	0.00
Blank	0.02

(1967), found between 10^8 and 10^9 non-methanogenic bacteria.ml^{-1} in an anaerobically fermenting sludge, and Hotchkiss (1952) obtained a count of 8 x 10^7 non-methanogenic bacteria per millilitre in the sludge of an Imhoff tank. Zeikus (1980) has reported levels of methanogenic bacteria in the range 10^6 to 10^8.ml^{-1} in sewage sludge digesters.

These results therefore indicate high levels of viable biomass (in agreement with those obtained by other workers) at a time when the digestion process in percolating packed bed reactors was in the steady phase of operation, that is to say when ATP concentrations in the liquid and solid phases are at steady levels, in addition to the liquor concentrations of volatile fatty acids and volatile solids.

5.4 Conclusions

1) The bed appeared to act as a filter for any solid particles which were entrained in the recirculating liquor. The average 2.13% dry weight of the liquor contained less than 2% particles coarser than 75μm. This is important as there was no build up of particles to block the recirculation line and a high proportion of the viable biomass is retained in the solid matrix.

2) Hydrogen sulphide content of the biogas was found to be initially high, but fell rapidly to around 250ppm. This was probably due to an easily degradable sulphur containing compound being present. It may however contribute to the inhibition of

methanogenesis in the early stages of digestion, but will also help in the establishment of anaerobic conditions.

3) Colonisation of the solid matrix was rapid, and after 10 days of operation over 90% of the biogas emanated from the solid matrix. However the liquor phase accounted for approximately 15% of the methane produced. This is possibly indicative of a slower colonisation of the solid matrix by methanogens compared with its colonisation by the other groups of bacteria present.

4) Estimation of adenosine triphosphate also showed the rapid colonisation of the solid matrix, with an almost linear increase in concentration, with a steady level being obtained after around 18 days of operation. ATP concentration in the latter stages of digestion was approximately constant. This possibly indicates a dynamic microbial population where rates of growth and attachment and rates of death and detachment are very similar leading to approximately constant biomass levels.

5) Microscopic analysis tends to confirm the above, showing a rapid bed colonisation, particularly of the straw fibres which have been degraded and deformed by the action of cellulases.

6) A number of specific bacterial species were indicated by electron microscope photographs, for example Methanosarcina and Methanospirillium species. Positive identification of these and other bacteria present cannot be made solely by microscopic analysis.

7) Viable counts of the liquor bacteria showed approximately 10^6-10^7/ml methanogenic bacteria and 10^7-10^8/ml non-methanogenic bacteria, and is of the same order with the findings of other workers examining the bacterial loads of anaerobic digesters.

Figure 5.1 Liquor Particle Size Distribution

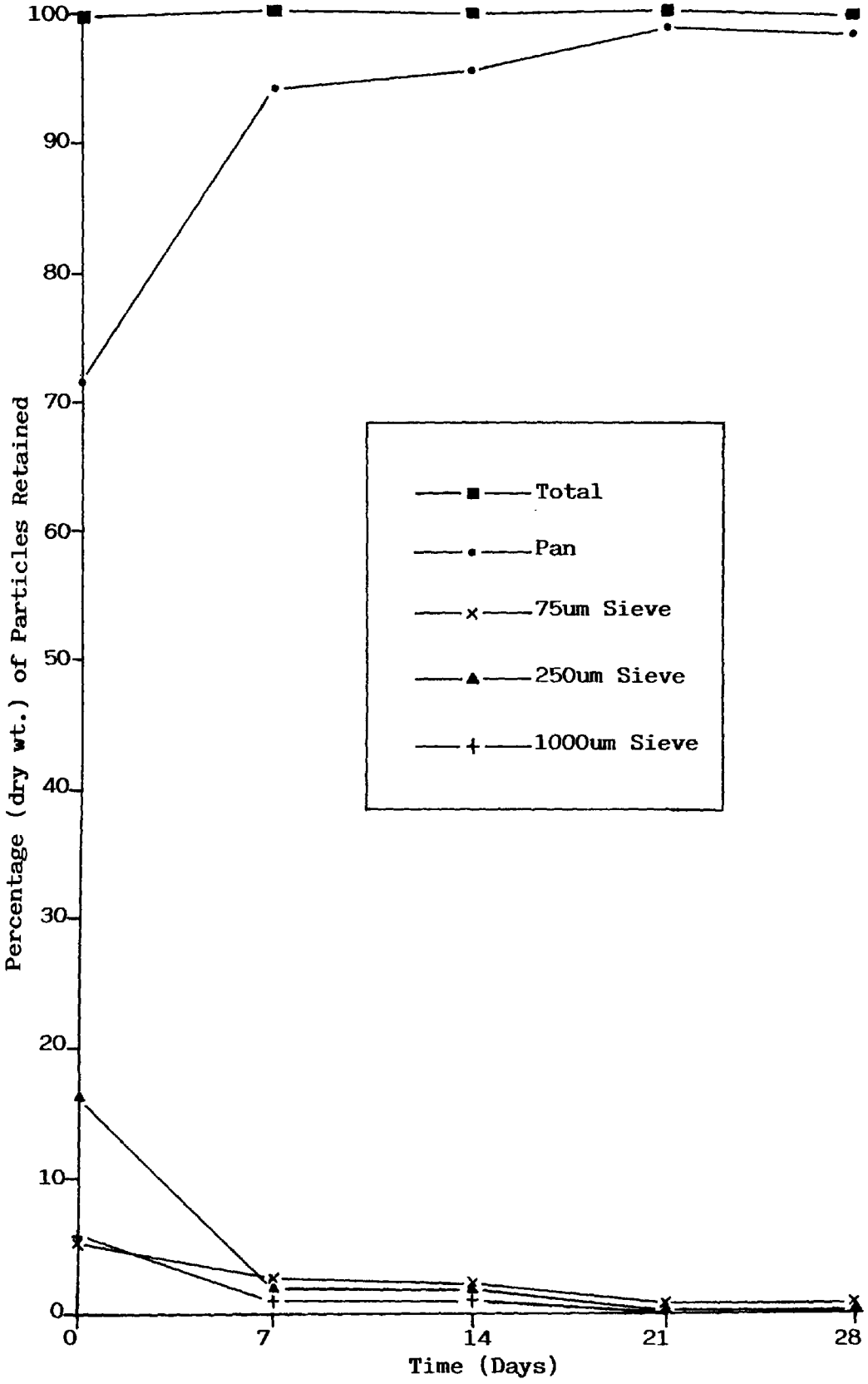
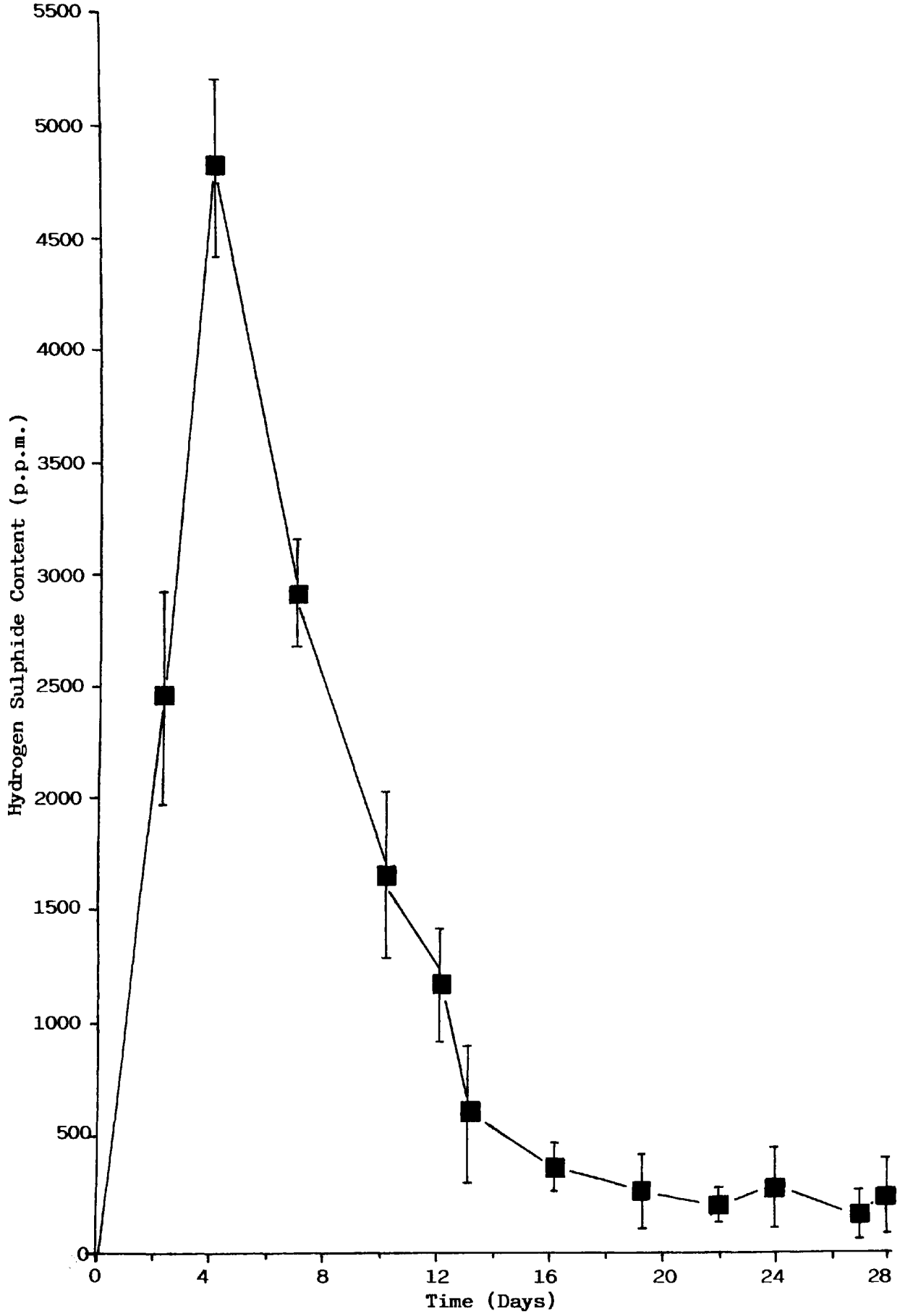


Figure 5.2 Biogas Hydrogen Sulphide Content



Mean values plotted, bar indicates Standard Deviation.

Figure 5.3(a) Total Daily Biogas Production

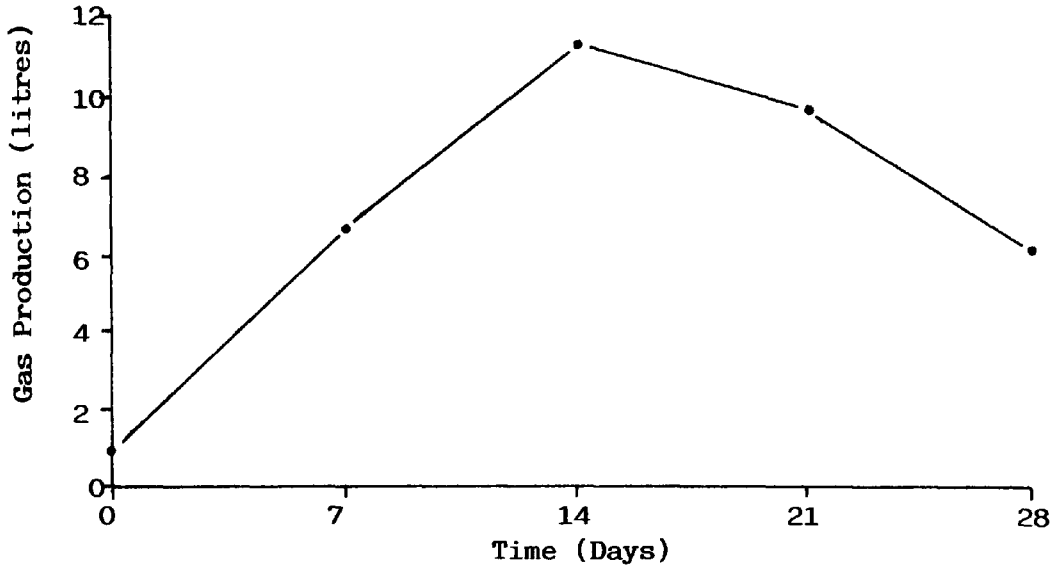


Figure 5.3(b) Proportional Biogas Production from the Solid and Liquid Phases

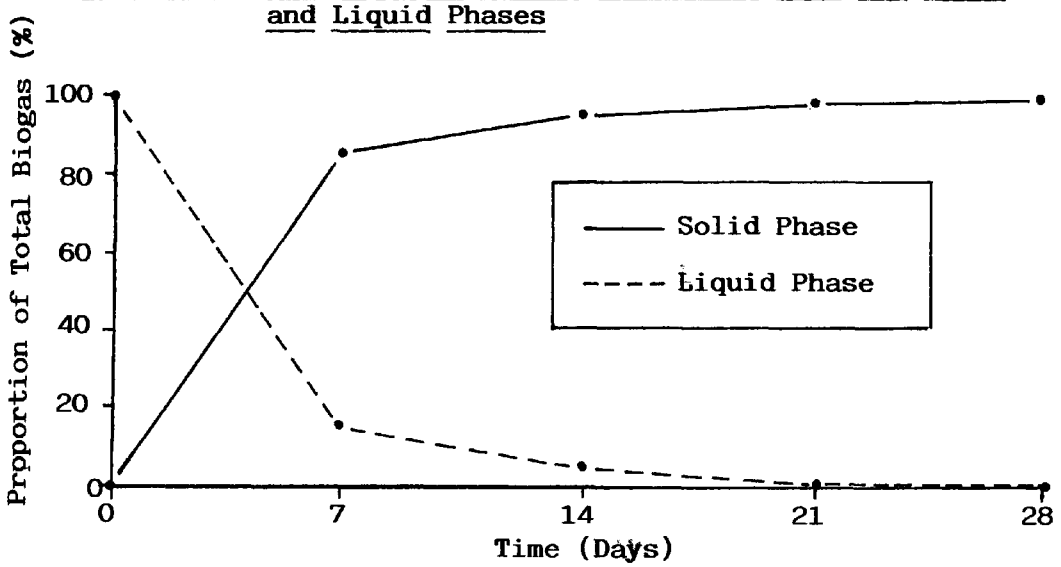


Figure 5.3(c) Methane Content of Biogas

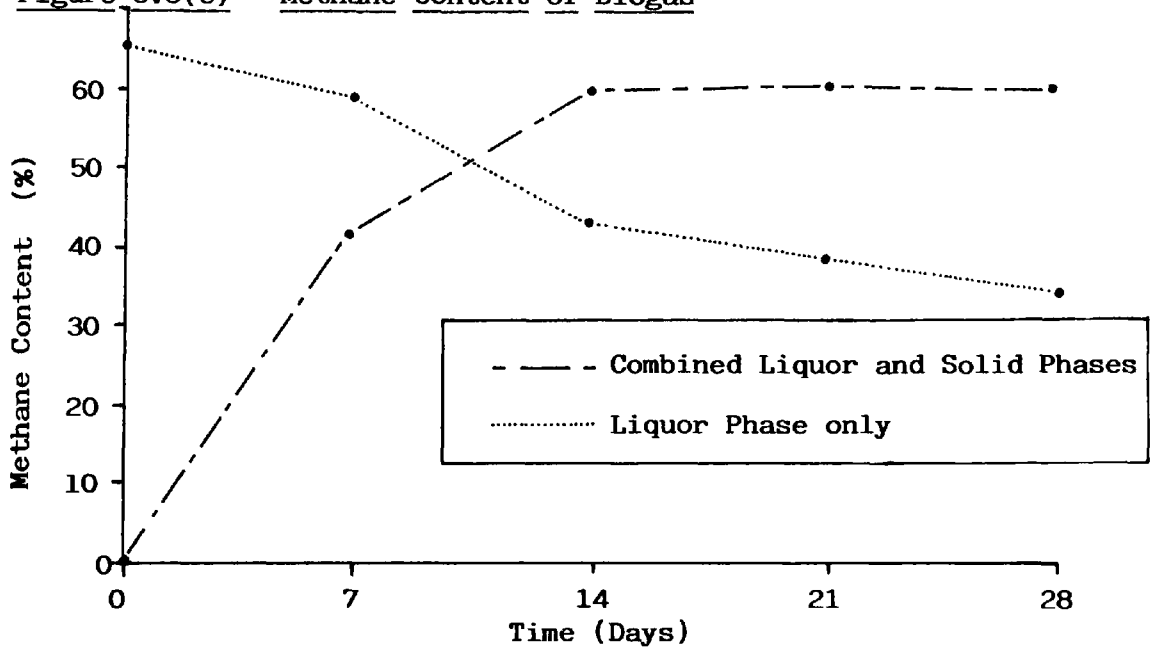


Figure 5.4 Adenosine 5'triphosphate Levels in the Solid and Liquor Phases

Mean values plotted, bar indicates range of results.

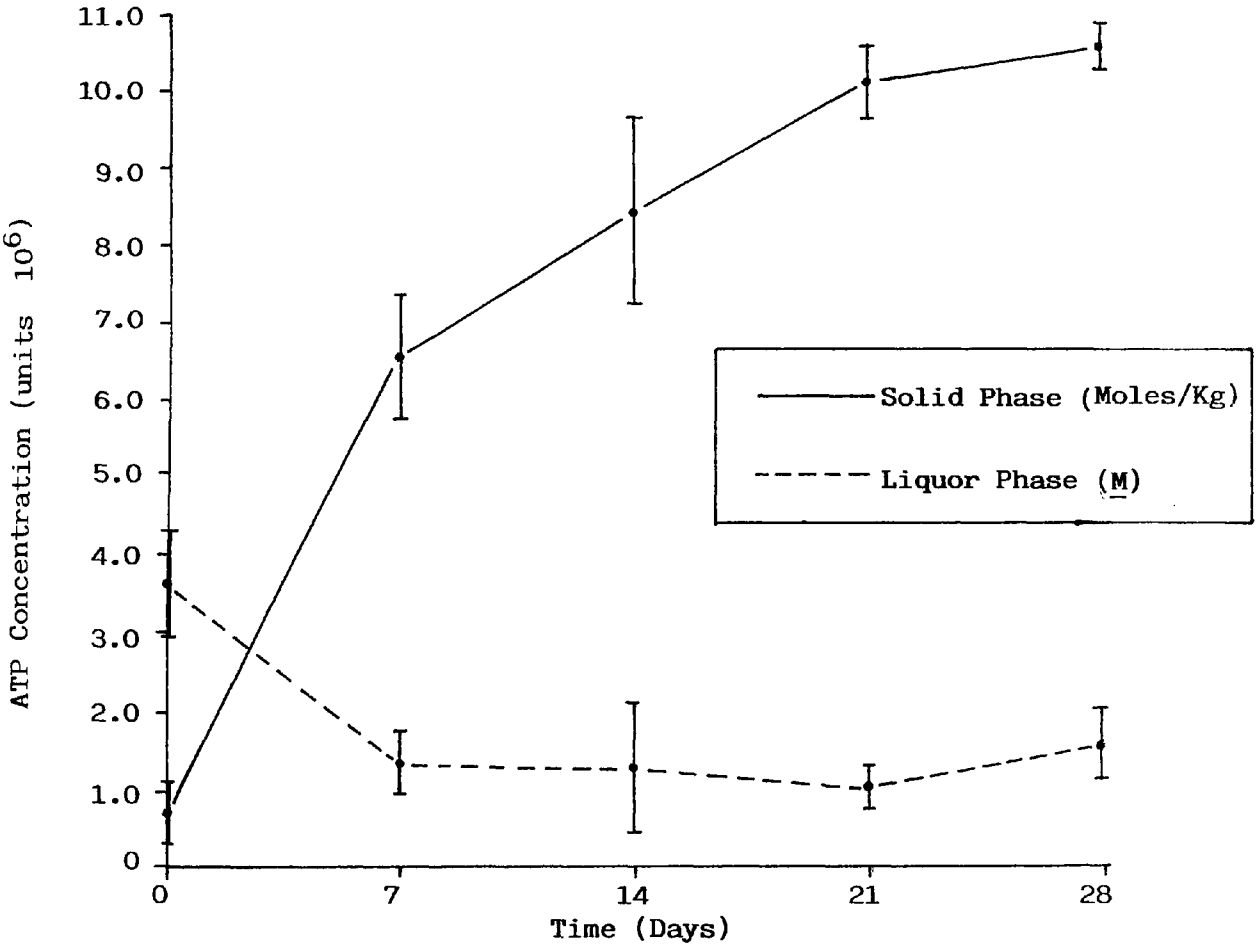
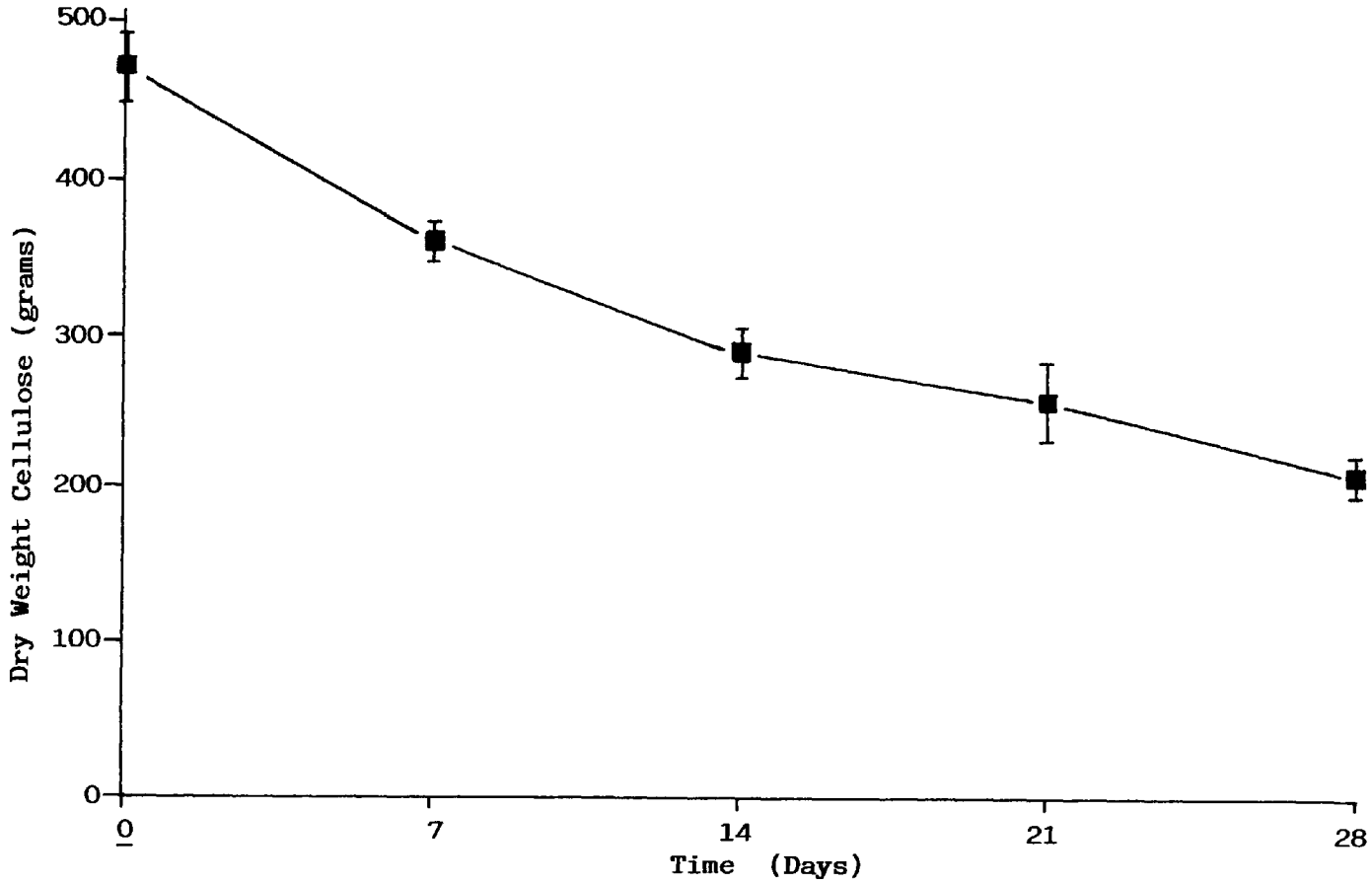


Figure 5.5 Solid Phase Cellulose Content



Bar indicates Standard Deviation

Figure 5.6 Results of Headspace Gas Analysis used in the Estimation Estimation of Methanogenic Bacterial Numbers

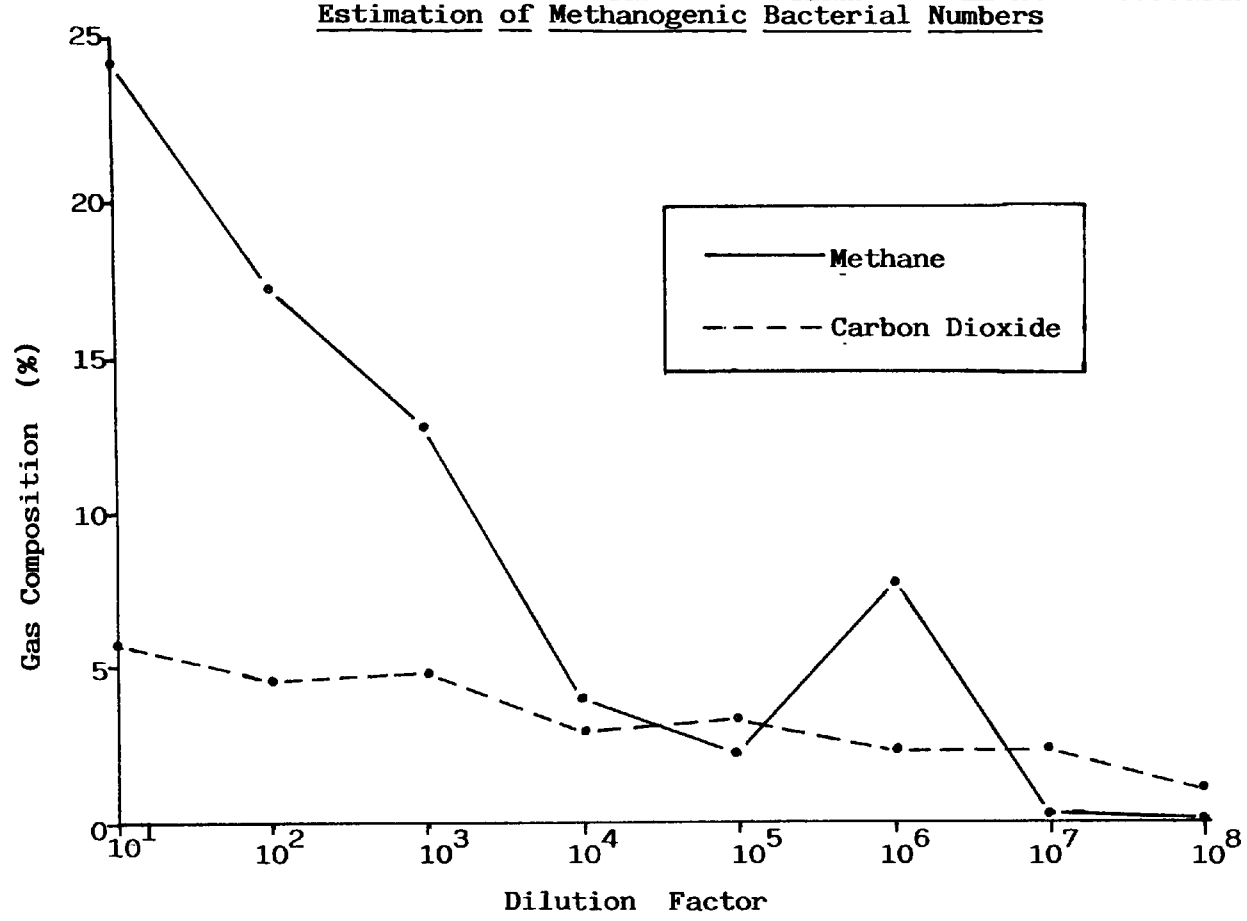


Figure 5.7 Results of Spectrophotometric Analysis used in the Estimation of Non Methanogenic Bacterial Numbers

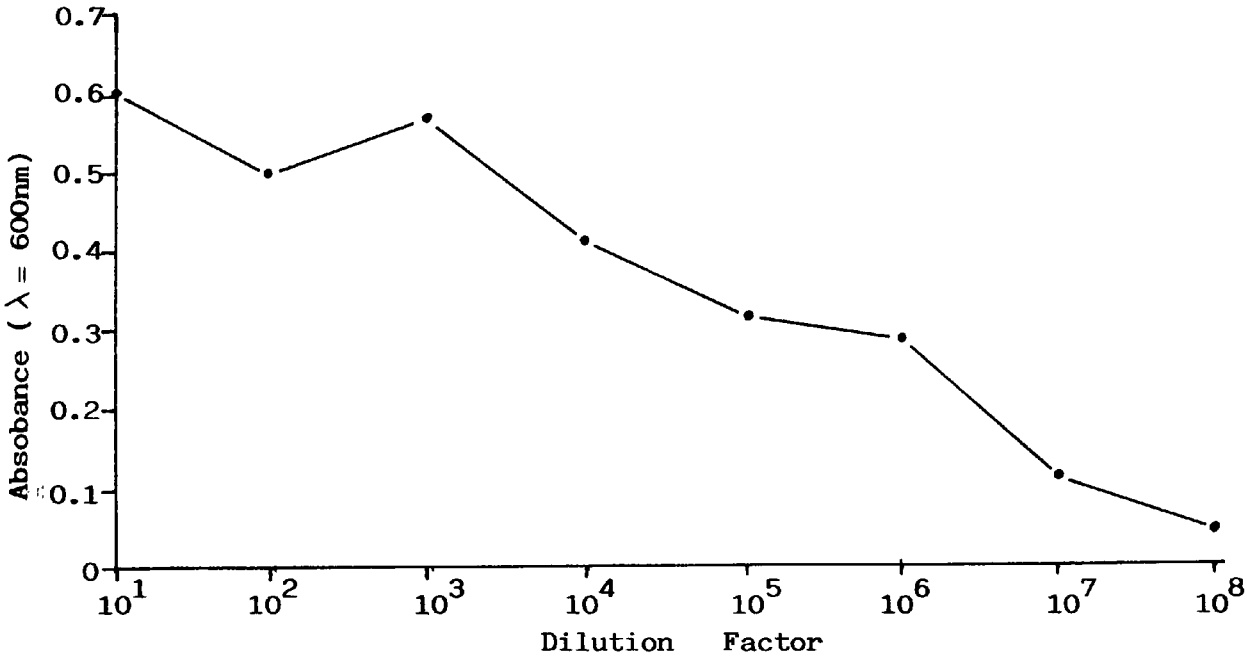


Plate 5.1.a. Initial Bacterial Colonisation of Straw Fibres in the Solid Phase

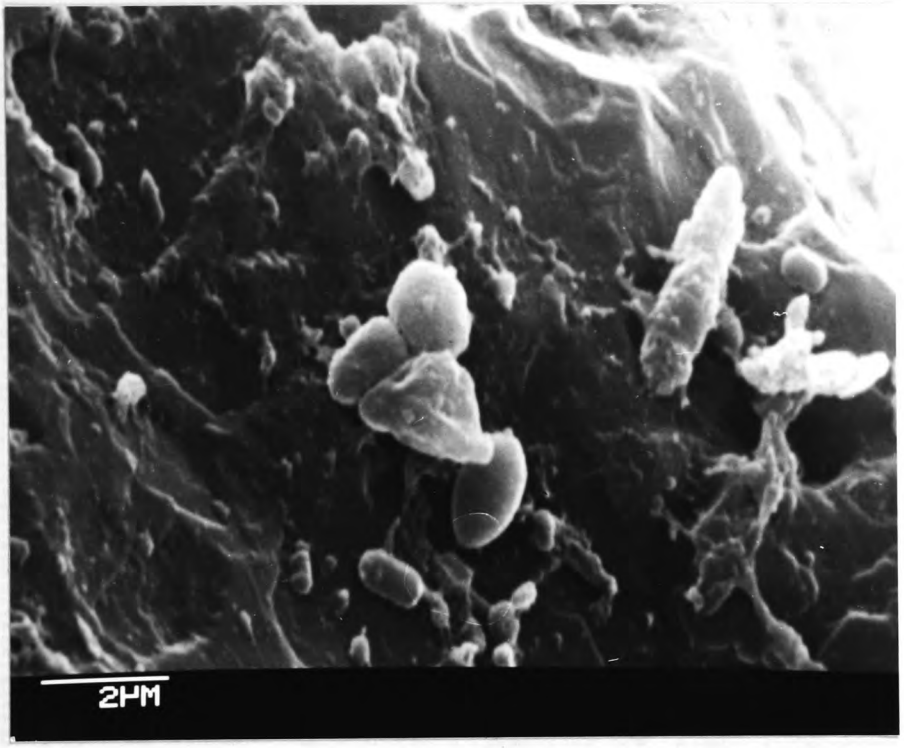


Plate 5.1.b. Initial Bacterial Levels within the Inoculum

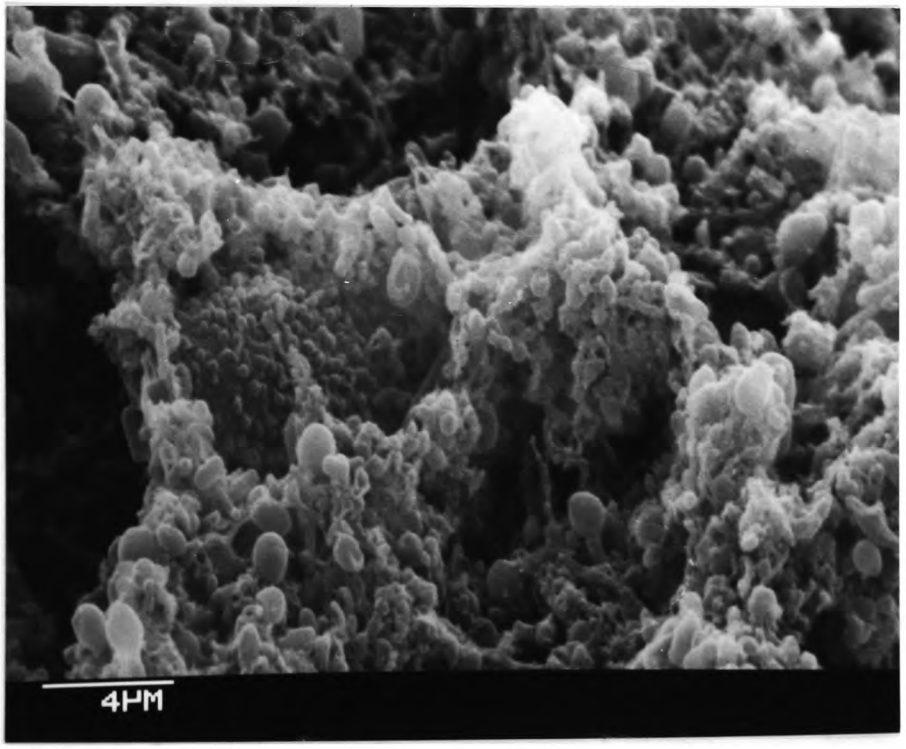


Plate 5.2.a. Colonisation of Straw Fibres by Cellulolytic and Non Cellulolytic Bacteria after 7 days of Operation

Plate 5.2.b. Reduced Liquor Biomass level after 7 days of Operation

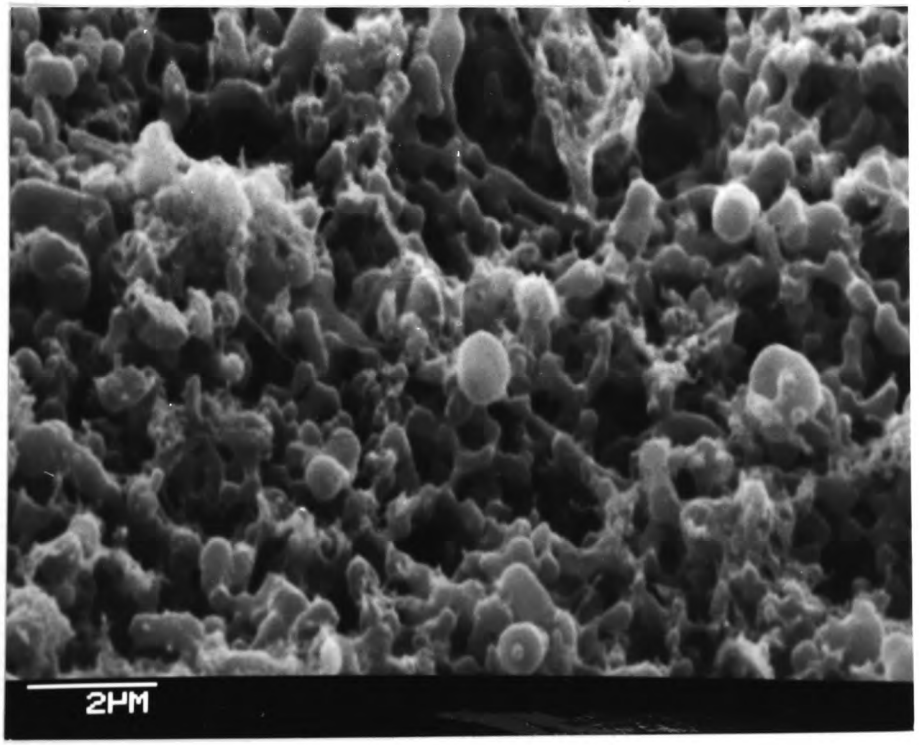


Plate 5.3.a. Straw Fibres after 14 days of Operation

A: Probable action of cellulolytic bacteria

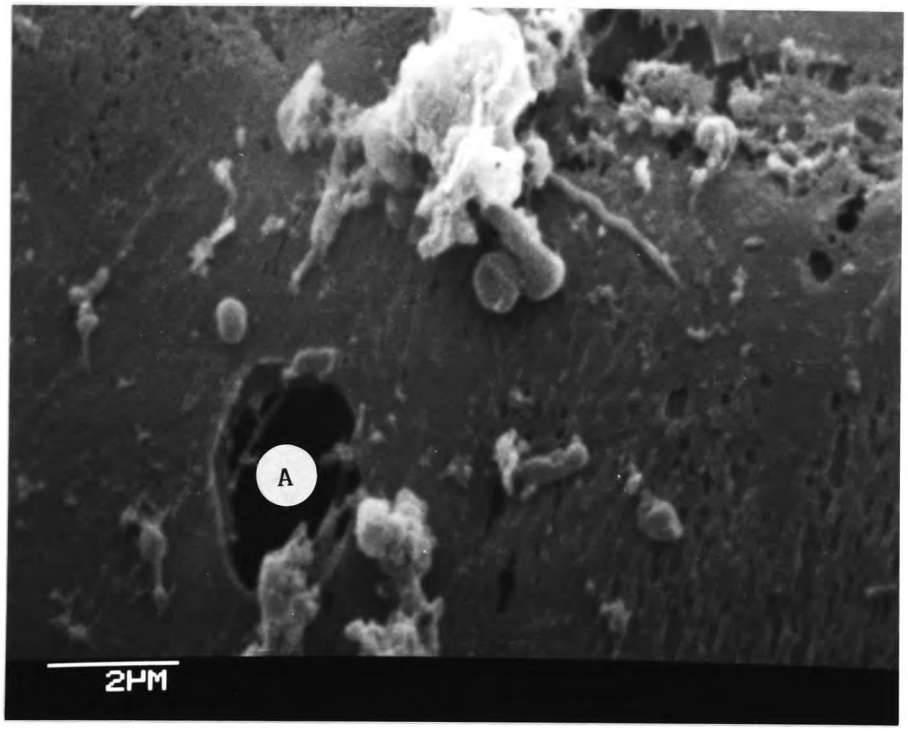


Plate 5.3.b. Liquor Biomass level after 14 days of Operation

A: Large Cocci similar in appearance to Methanococcus vannielli

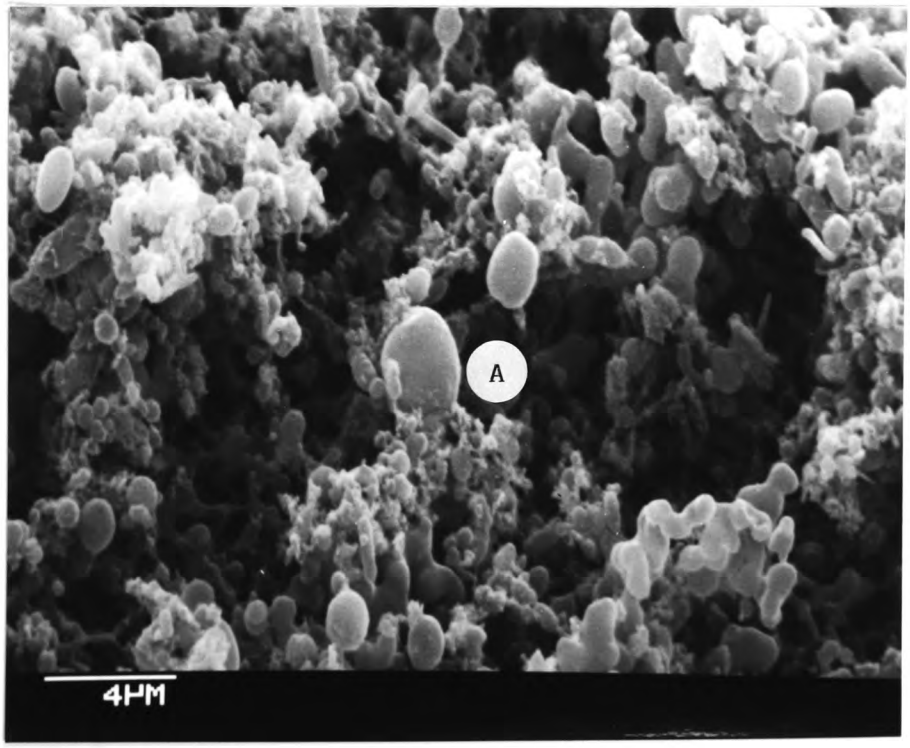


Plate 5.4.a. Straw Fibres after 21 days of Operation

A: Methanosarcina like species

B: Methanospirillum like species

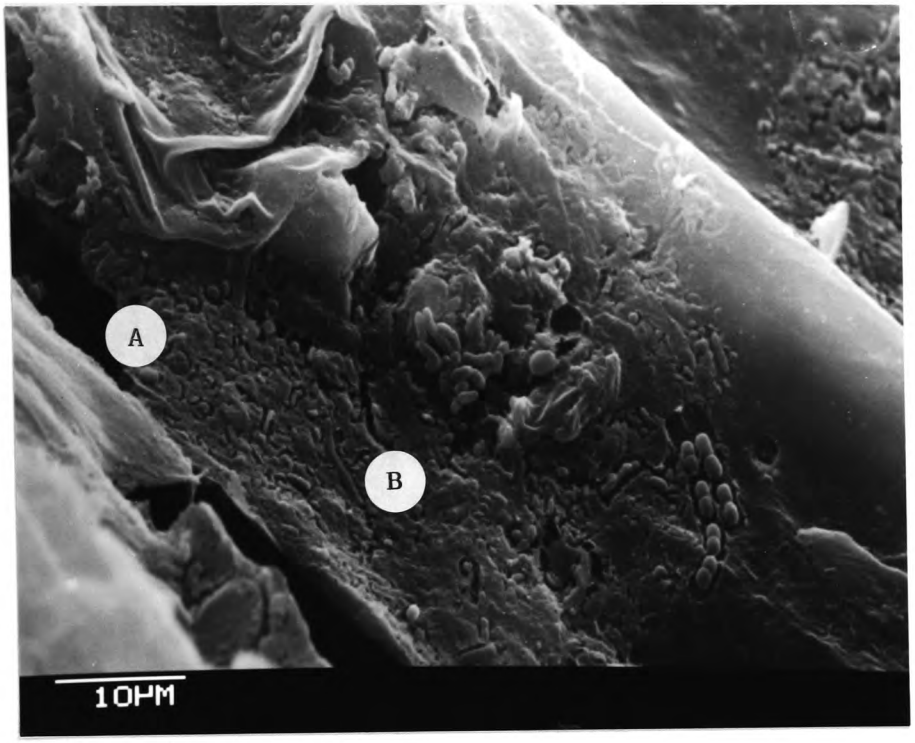


Plate 5.4.b. Results of attack by Cellulases after 21 days of Operation

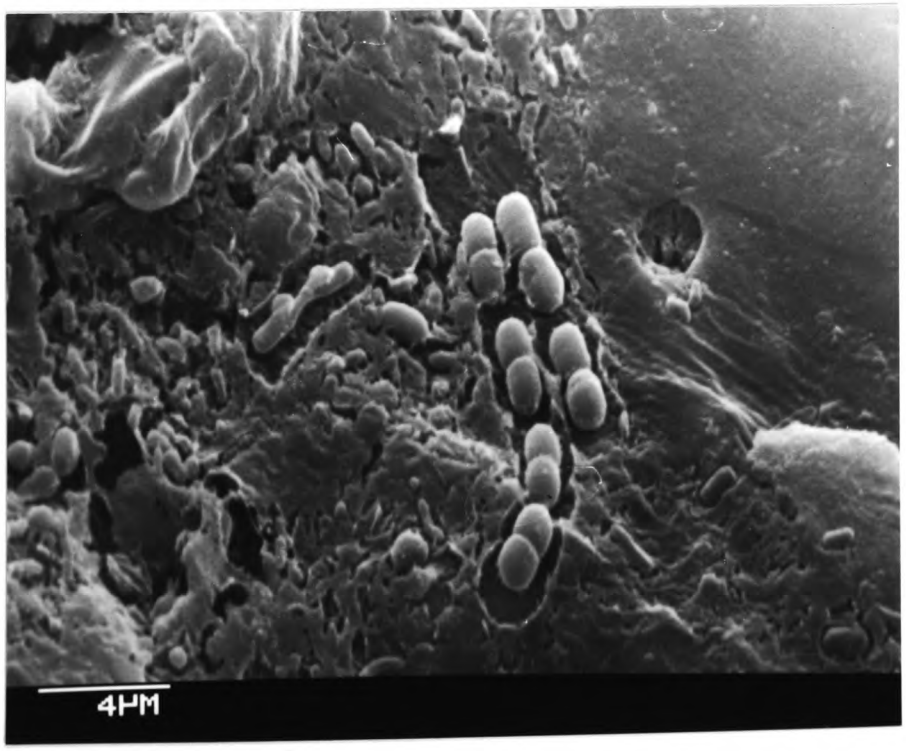


Plate 5.4.c. Non Cellulolytic Bacteria attached to roughened areas of the Straw Fibre after 21 days of Operation

A: Methanobrevibacter like species

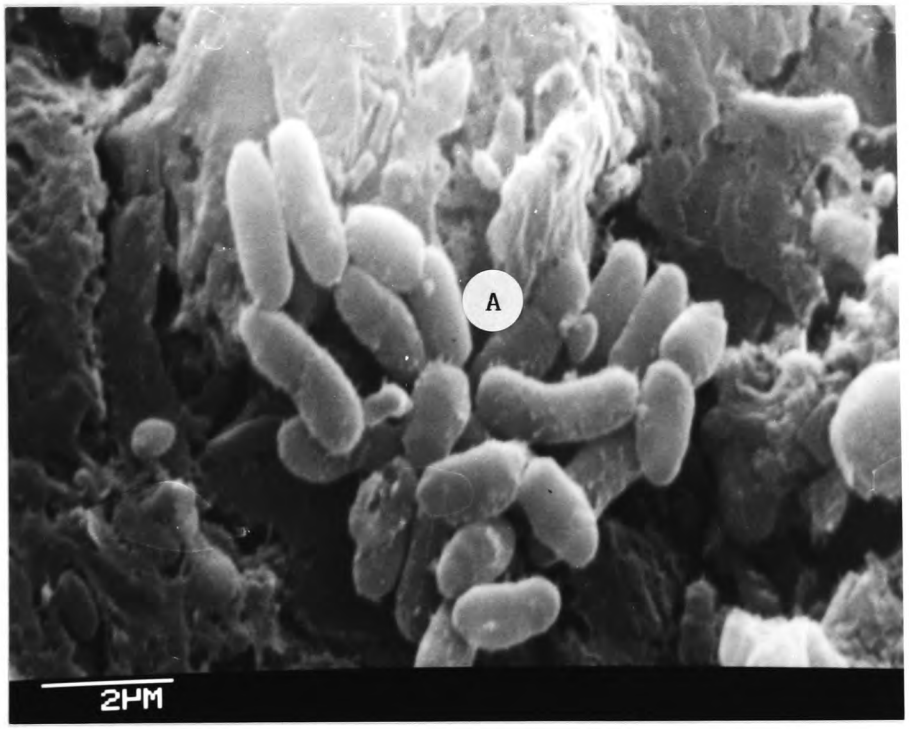


Plate 5.5.a Highly Irregular Straw Fibre Surface Caused by the Action of Cellulolytic Bacteria after 28 days of Operation

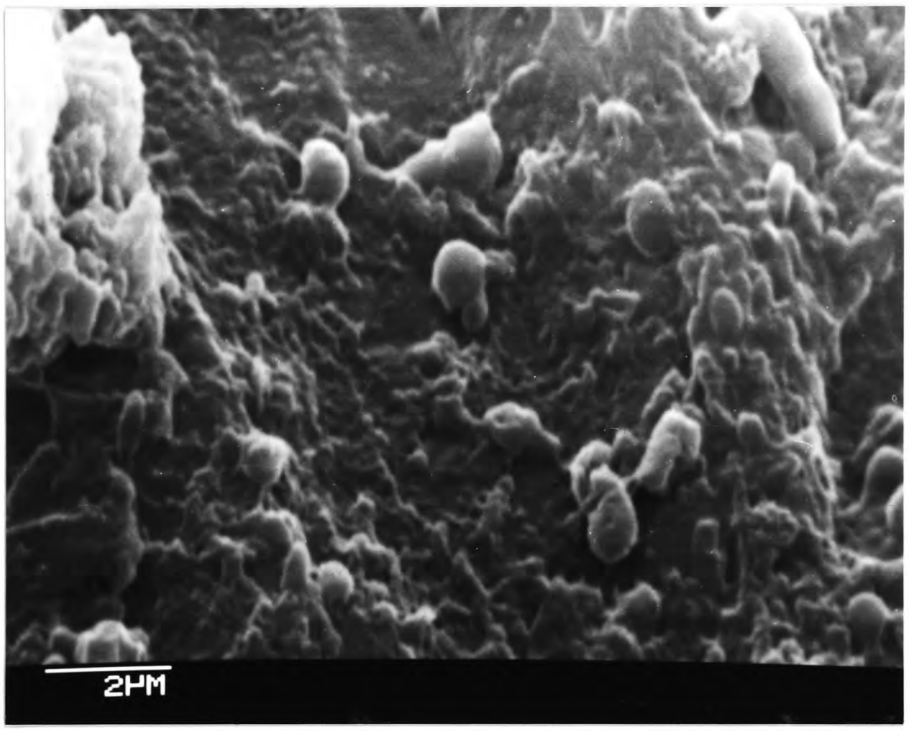
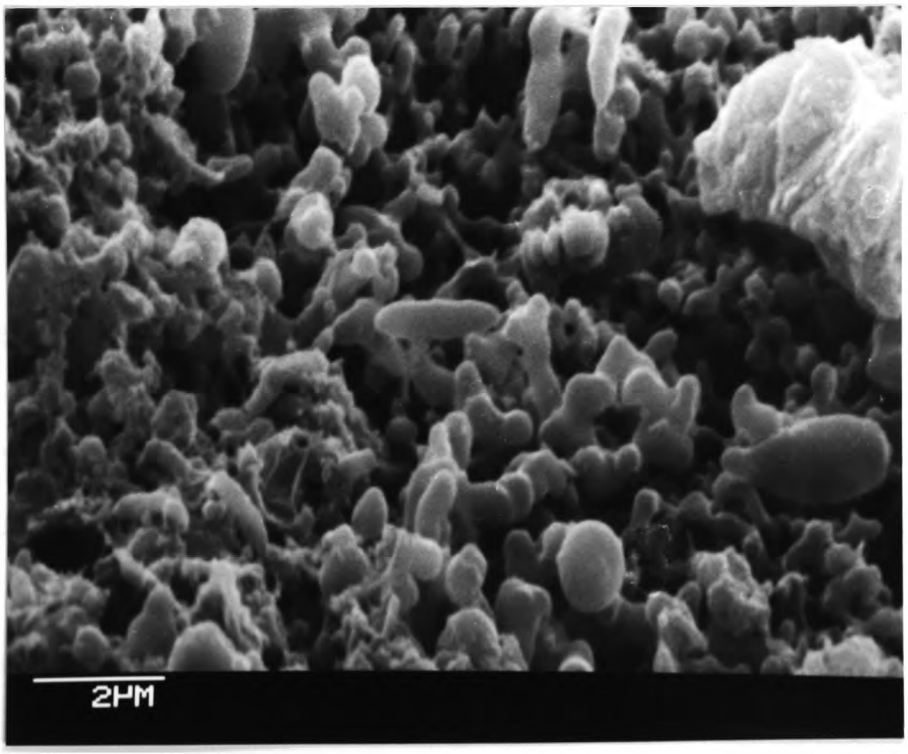


Plate 5.5.b Liquor Biomass Levels after 28 days of Operation



CHAPTER 6

FEASIBILITY STUDY - SEMI-CONTINUOUS PROCESS

6.1 Introduction

The experiments and results described in Chapters 3 and 4 were required to optimise batchwise anaerobic digestion of high solids cattle waste, and to elucidate any technical problems inherent in the system as such before a semi-continuous mode of digestion could be studied experimentally. Semi-continuous operation of this type of digester might prove to be highly beneficial, in particular as an added possibility for the digestion of waste occurring over an extended period of time (e.g. in this case over the whole winter period). Further, continous operation should be investigated in its own right to evaluate any potential advantages over batch operation.

The principle objective of this study was the feasibility and performance of semi-continuous anaerobic digestion of high solids dairy manure-straw mixtures in percolating packed bed digesters. That is the linking of fresh digesters through their recirculation systems to partially expended digesters (Section 2.3.2). This mode of operation would be more likely to lead to problems involving the accumulation of inhibitory compounds since it is a more complex system. The initial study could therefore be used to measure the extent of any such difficulty and allow them to be minimised. The feasibility study would also ascertain if for

example the system would require re-inoculation of each stage of operation, and whether the efficiency of the process measured by its gas yield is improved by the development of a semi-continuous system.

6.2 Digester Operating Conditions

Experiments described in this feasibility study were conducted in duplicate and the figures presented are averages of all runs. The experiments utilised 3 kg wet weight of solid substrate and to the initial digester 3 litres of liquor was added (obtained as described in section 2.2.4) which provided the inoculum. The liquor was recirculated at a rate of 3 litres/hr and the operating temperature was 30°C. During the semi-continuous mode of operation a second un-inoculated digester containing fresh substrate was linked to the initial digester after it had operated for 40 days via their recirculation systems. No new inoculum was added, 3 litres of deionised water was used as the new circulation liquid for each unit added subsequently. The digesters were then operated in series for a further 30 days before the experiments were terminated and the final solids analysed. The experiments were carried out under conditions dictated by the findings of batch experiments. However as this feasibility study was conducted before the optimisation of batch digesters had been completed, the actual conditions may not strictly reflect the findings of the batch process optimisation programme.

6.3 Digester Monitoring

The aspects of digester feeding, monitoring and sample analysis have been previously described (Chapter 2). The feed composition used in these experiments is described in Table 6.1

Gas production and composition together with internal digester temperature was monitored daily. Constituents of the liquor phase were monitored as follows:- Volatile fatty acids, pH, ammoniacal nitrogen, total nitrogen, total solids and volatile solids were monitored twice weekly. Alkalinity was determined weekly.

Table 6.1 Substrate Composition used in the Linked Digester Programme

Total Solids (% wet wt.)	26.64	(±0.32)
Volatile Solids (% TS)	80.40	(±1.10)
Volatile Fatty Acids (ppm)	2450	(±376)
Total Nitrogen (ppm)	6774.5	(±452)
Ammoniacal Nitrogen (ppm)	1486.5	(±387)
Cellulose (% wet wt.)	14.28	(±0.30)
Lipids (g/kg)	9.5	(±1.38)

6.4 Results and Discussion

Digesters were found to operate without problems when operated in series. There was no inhibition due to the build up of toxicants or any blockages occurring. Linking of digesters was rapidly accomplished by simply re-attaching the recirculation system from the initial digester to the second digester and replacing with that of the second digester. The silicone rubber tubing of the recirculation systems of both digesters ran through the same peristaltic pump.

6.4.1 Gas Production and Yield

Gas production from the initial reactor started immediately upon achieving anaerobic conditions, and followed the same trends observed in the feasibility study of batch processes. The same operating conditions were used, and any minor variations were a result of compositional differences in the substrates used.

The second digester was then linked to the first after it had been operational for 40 days. The results are shown in Figure 6.1. Gas production from the first digester increased immediately upon linking to approximately 5 litres/day, and remained at this level for a futher 6 days. The rate of gas production then decreased more rapidly than for a batch digestion, dropping to 1.75 litres/day after a further 6 days and then continuing to decrease to approximately 1 litre/day after 70 days of operation.

Gas production from the second uninoculated digester followed a similar trend to that which would be obtained in a batch process described previously. High levels of gas production occurred immediately. There was no lag phase in contrast to single unit operation. However, the period of high rate gas production (approx.4.5 l/day) lasted 6 days compared with an average of eight days in single unit operation.

It would appear from these results that dissolved or suspended nutrients washed from the bed of the second digester into the recirculation stream and are thus transfered to the initial digester whose attached flora digests the transfered nutrients, thus increasing gas production from the initial digester. The initial digester thus acts in a similar manner to a fixed film reactor treating soluble waste (Koster, 1984). At the same time bacteria, which have been shown previously to be present at substantial levels are transfered via the recirculation system to the second digester, thus providing its inoculum. Gas production began immediately, partly due to bacterial numbers and well adapted nature of the seed which has been shown by Hills (1980a) to greatly influence the efficiency of start-up in batch processes. These factors will have important implications to the efficiency and viability of this type of treatment process, and are discussed later.

While a gas yield of approximately 0.21 m^3/kg VS added could be expected from batch digesters operating over a 70 day period, a figure of 0.264 m^3/kg VS added was actually obtained from the first

digester. The gas yield from the second digester was 0.15 m^3/kg VS added after 30 days of operation which was comparable to that of a batch digestion operating for a 30 day period. If these results are translated into cumulative gas production, a combined average of 241.9 litres was obtained. While from two batch digesters (one operating for 70 days and the second for 30 days) a value of 205 litres could be expected. Semi-continuous operation thus increases gas production by approximately 18%.

The semi-continous mode of operation thus substantially increases gas production, primarily by rapid inoculation of the second digester, and a transfer of nutrients to the initial digester.

6.4.2 Gas Composition

Gas composition reached its maximum level after 6 days in the initial digester (61.7% ± 20%), and remained at this level throughout the course of digestion. Methane content of the biogas from the second digester reached its maximum level of 61.1% (±2.1%) after only 4 days of operation. This was reflected in the VFA content of the liquor (see section 6.4.3) and was partly a result of well adapted inoculum, and the treatment of metabolites by the initial digester acting as a filter.

6.4.3.1 Liquor Volatile Fatty Acid Concentration

Liquor volatile fatty acid concentration (Fig.6.2) for the first digester shows an inital rapid rise reaching a maximum concentration of around 4000 ppm after 3 days followed by an equally rapid decline to around 1750ppm. VFA concentration then fluctuated apparently randomly around a steady concentration of on average 1584 (±120) ppm, even after the second digester was linked to it.

Liquor volatile fatty acid concentration of the second digester did not reach inhibitory levels (maximum 2000ppm) even over the first few days of digestion, and was shown in the rapid rise in gas production with no lag phase and the rapid attainment of maximum methane content of the biogas. The increased gas production from the initial digester on addition of the fresh digester is explained by the slightly increased VFA levels (approximately 2000ppm) leaving the second digester to be treated by the first.

Volatile fatty acid concentrations within the liquor phase were approximately 300ppm lower than in the solid phase, as was determined by the examination of both liquor and solid phases at the end of the digestion period. The average solid phase concentration was found to be 1920 (±186) ppm. It is probable that as hydrolysis and acid production take place within the solid matrix, VFA's are treated by bacteria attached to the solid matrix; a proportion however is carried through into the recirculation

stream.

During the start-up of batch digesters, the liquor VFA concentration reached inhibitory levels and hence was inhibitory in the solid matrix. However, during semi-continous operation, liquor VFA concentrations entering the fresh digester are low (approximately 1700 ppm) and thus non-inhibitory. In addition the liquor VFA concentrations leaving the second digester (approximately 2000 ppm) indicate a solid phase concentration in the region of 2300 ppm. This was of great importance as these concentrations are not inhibitory, and are indeed of the order which will stimulate gas production (Stafford, 1982). Thus start-up of the fresh (second) digester occurs immediately, and gas production was stimulated in the initial (first) digester.

6.4.3.2 Liquor pH and Alkalinity

Liquor pH again follows the VFA concentration (Figure 6.2) and in the initial digester decrease to 7.05 after 5 days of operation. This was followed by a rapid increase, and had an average value of 7.52 from day 12 onwards. Liquor pH of the second digester remained approximately constant throughout the digestion period having an average value of 7.52 (±0.10). Alkalinity was consistently high at all times being an average 6516 (±429) mg/l of Calcium carbonate. The system was thus highly buffered, minimising pH fluctuations, allowing equilibrium to be rapidly developed and preventing the possibility of digester souring.

6.4.4 Solids Loss

Analysis of the solids phases after digestion showed the following solids losses:-

	Operation period	T.S. loss	V.S. loss	Cellulose loss
Initial Digester	70 days	27.0%	31.9%	56.8%
Fresh Digester	30 days	24.8%	27.1%	30.7%

The results show similar levels of solids losses in the initial digester to that of a 70 days batch digestion. Solids losses from the second digester are approximately 20% greater than for a batch digestion over the same period, however its gas yield was very similar to that of a batch digestion. During semi-continuous operation the gas production of the initial digester was increased and this appears to be as a direct result of the increased solids losses from the second digester. This was due to soluble material being washed from the second digester and treated by the attached flora of the initial digester, and a well adapted flora being used to inoculate the second digester, thus preventing the high volatile fatty acid levels and inhibition of methanogens. The increased solids losses are particularly noteworthy as they will considerably reduce the volume of final waste and hence its handling costs. Much of the beneficial action of a well adapted seed can be accounted for by cellulose degradation. Hobson *et al* (1981) reported that straw was not degraded in a conventional digester at retention times below 20 days due to inappropriately acclimatised bacteria. The use of mechanically chopped straw would

make more cellulose available for digestion and hence increase volatile solids loss and gas production (Hills & Nakano, 1984). The economics of this for farm scale digestion are however not clear and careful evaluation of the increase gas production and the energy required for chopping the straw would need to be considered on a laboratory scale before they were implemented in a full scale process.

6.4.5 Effect of Digestion on Nitrogen concentrations in the liquid and solid phases

The liquor concentration of both ammoniacal and total nitrogen (Fig.6.3) showed an increase in concentration of similar proportions to that of a batch process (section 3.4.7), for both the initial and fresh digester. Over a 70 day digestion period the liquor ammoniacal nitrogen increased by on average 307ppm from an initial level of 358 (±39)ppm, and total nitrogen increased by on average 351ppm from an initial level of 858 (±61)ppm. There was thus an increase of approximately 44ppm in non-ammoniacal nitrogen due most probably to an increase in lignin masked nitrogenous material. Nitrogen levels of the liquor emmanating from the initial digester appeared to be unaffected when the second digester appeared to be unaffected when the second digester was added, and continued to increase slightly throughout the course of digestion (Fig.6.3).

The level of nitrogenous material in the liquor from the second digester (where only water was added), rapidly attained

levels similar to that of the first digester. The concentrations of both ammoniacal and total nitrogen emanating from both digesters remain closely similar to the end of the digestion period. This was as would be expected, as the nitrogen present does not play a direct role in the digestion process and therefore an equilibrium will be rapidly established in the liquor of both digesters.

The nitrogenous material is present in proteins, bacterial mass, free in solution and in recalcitrant compounds. Its turnover appears to be small and approximately constant as indicated by the steady increase throughout the course of digestion. Nitrogen loss from the system was low and increased liquor concentrations are due to material leached from the solid matrix.

The ammoniacal nitrogen levels found, of up to 700ppm were not inhibitory, McCarty and McKinney (1961) quoting levels of over 3000 ppm to be toxic, though Van Velsen (1979) has shown that with acclimatisation, higher levels can be tolerated. Wiegant & Zeeman (1983) have suggested that ammonia affects the specific growth rate of hydrogen consuming methanogens leading to an increase in propionic acid which will inhibit acetate breakdown.

The loss of nitrogenous material from the solid phase of the initial digester over a 70 day operation period was 452 ppm ammoniacal nitrogen and 748 ppm non-ammoniacal nitrogen. The fresh digester operating for 30 days showed losses of 437 ppm ammoniacal nitrogen and 113 ppm non-ammoniacal nitrogen. It would

thus appear that the loss of ammoniacal nitrogen occurs immediately upon start up and was of a finite level dependent on the waste used, and was probably unattached in the solid matrix. Non-ammoniacal nitrogen however was lost from the bed in a progressive manner probably by the degradation of nitrogen containing polymers.

Losses of nitrogenous material from the initial digester were found to be greater than those obtained from a batch process over the same time period (70 days) under the same operating conditions. No clear explanation exists for this fact, however the increased availability of liquor occurring when the second digester was added may be an influencing factor.

The loss of total nitrogen does not constitute a significant loss of fertilising potential of the waste, as all but 7.2% was transfered to the liquor phase.

6.4.6 Solids Content of the Liquor Phase

The volatile solids content of the dry matter contained in the liquor dropped from an initial level of 71.49% to approximately 30% in 19 days (Fig.6.4) in a similar manner to that previously described (Section 3.4.8). It then remained approximately steady at an average concentration of 29.8% until the second digester is added. The V.S. concentration of the liquor from the first digester then increased to approximately 40% of the dry matter content. Over the next 30 days of operation this concentration

decreased to 30% of the dry matter content. The V.S. content of the liquor emanating from the second digester was initially high (over 60% of the dry matter content. This liquor was transfered to the initial digester, and accounted for its increased gas production.

The volatile solids content of the liquor from both digesters equilibrated and reached an approximately steady level 20 days after linking of the two digesters had been accomplished.

The total solids content of liquor from the initial digester remained approximately constant throughout the course of digestion, fluctuating between 1.16% and 1.76% with an average of 1.54%. Addition of the second digester caused the T.S. content of the liquor from the initial digester to drop to 1.16% for one day only, its total solids content did not drop below 1.41% at any other time during digestion. The decrease was due to water only being added to the second digester, the liquor of which had a total solids content on start-up of 0.94% and was transfered via the recirculation stream to the second digester and was thus reflected in the solids content of the liquor from this digester. The liquor of the second digester rapidly increased in dry matter content to give an average value of 1.57 (±0.12)%. The results are shown in Figure 6.4.

6.5 Conclusions

1) The operation of two digesters in series to form a semi-continuous process was found to function successfully and to have increased solids reduction and gas production when compared with batch digestions operating over the same period.

2) Gas production was increased by 18% over that of two batch digestions operating for 70 and 30 days respectively. The gas yield of the second digester was similar to that of a batch process, while that of the initial digester was increased. It is envisaged that this will occur on each occasion a fresh digester is linked in series.

3) Souring of digesters did not occur, and inhibitory levels of ammoniacal nitrogen (McCarty and McKinney, 1961) were not apparent.

4) Digestible material was transfered from the second digester on start-up via the recirculation stream to be treated by the attached flora of the first digester, thereby increasing its nett gas production. There was no build up of volatile fatty acids when the second digester was added and thus no inhibition of biogas production occurred.

5) The linking of digesters was simply accomplished with no noticeable effect of any oxygen which may have entered the digesters. No new inoculum was added.

6) The solids losses of the second digester were increased compared to a batch process, and are an improvement on those obtained by other workers (e.g. Wong-Chong, 1975). A greater volume reduction of solids will be obtained, thus easing problems of post-digestion handling of the waste.

7) The low losses of nitrogenous material which occur on linking, indicate it to have little effect on the fertilising potential of the waste. It appears that ammoniacal nitrogen loss occurs immediately upon start-up, whereas non-ammoniacal nitrogen was removed from the bed more slowly.

8) The results again showed that the volatile solids content of the liquor dry matter decreased over the course of digestion, and with it the rate of daily gas production.

9) It is envisaged that the linking of a number of digesters in series will not only increase gas production, but also equalise the rate of production to give a more even flow of gas to be used for heating, lighting or the generation of electricity.

Figure 6.1 Daily Gas Production from Semi-Continuously Operated Digesters

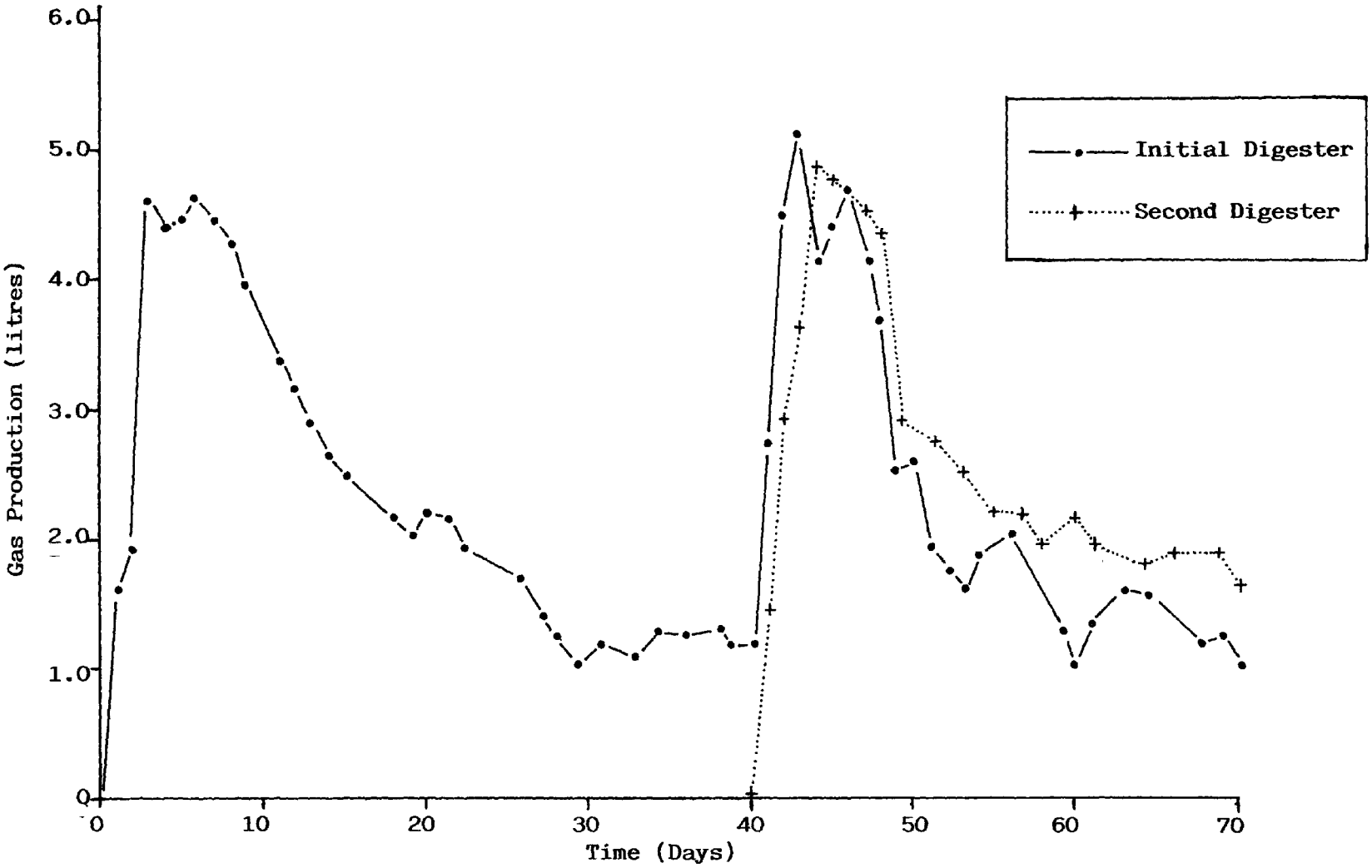


Figure 6.2 Variation in Liquor pH and VFA Concentration

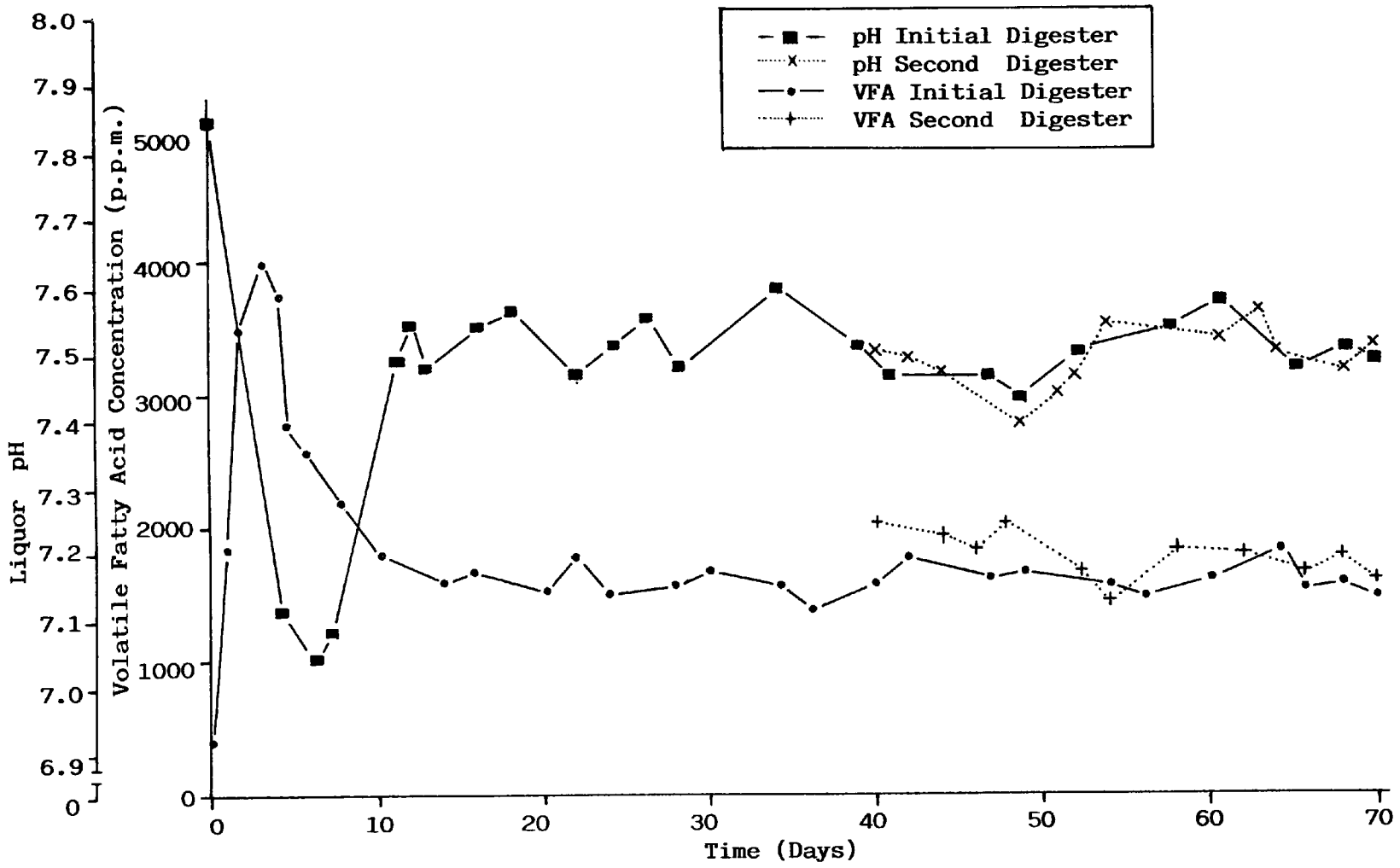


Figure 6.3 Increase in Liquor Nitrogen Content during Semi-Continuous Operation

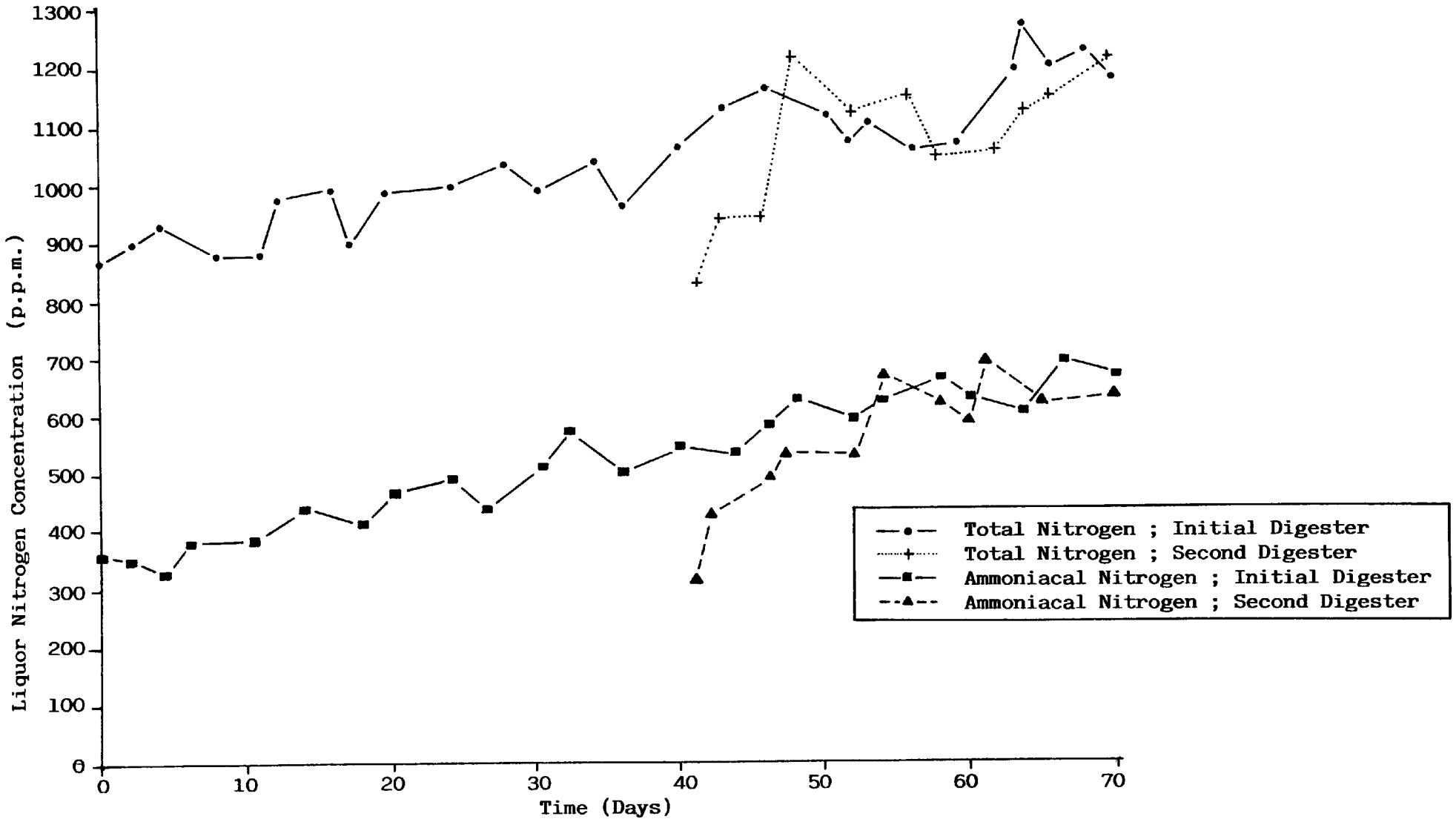
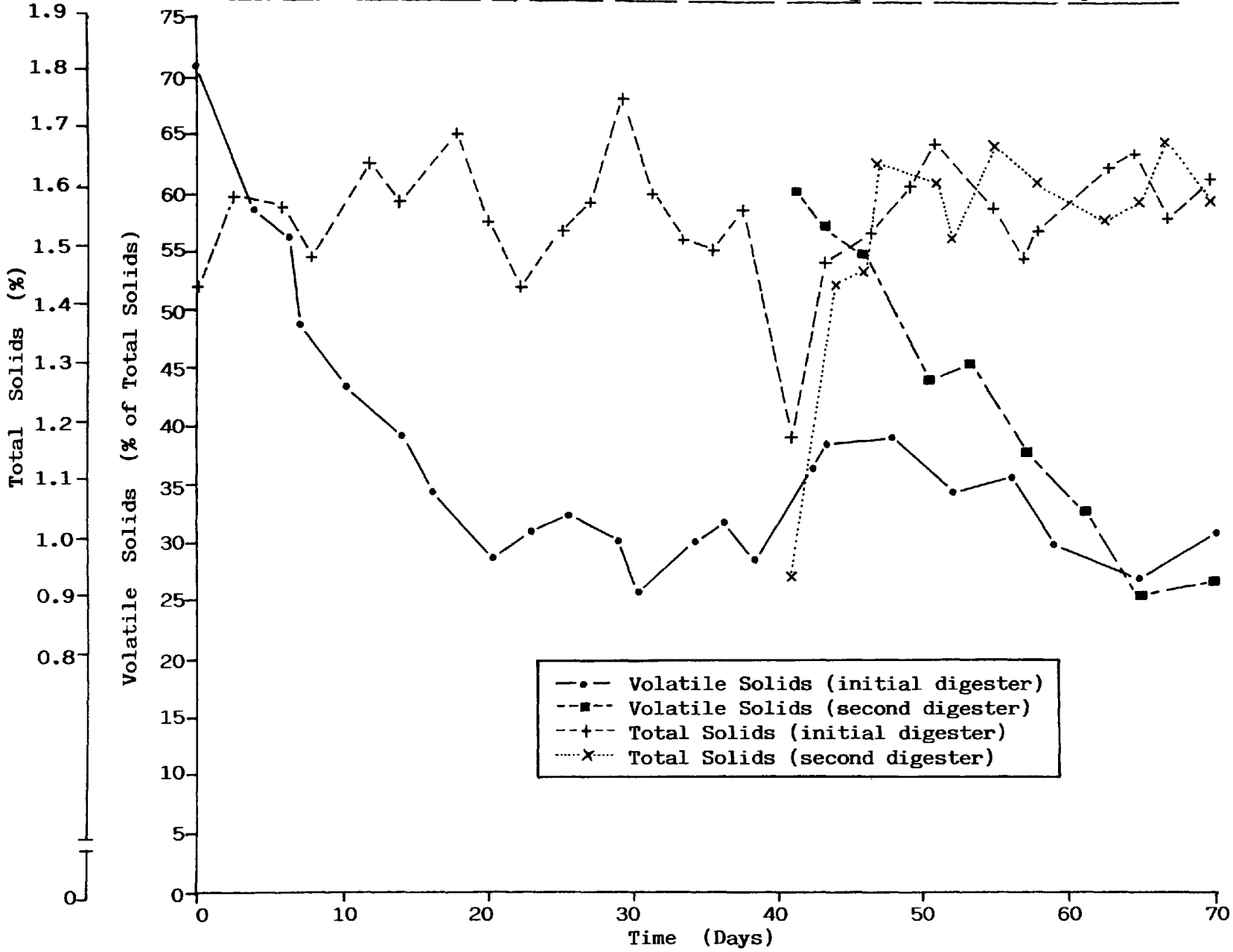


Figure 6.4 Variations in Liquor Solid Content during Semi-Continuous Operation



CHAPTER 7

OPTIMISATION - SEMI-CONTINUOUS PROCESS

7.1 Introduction

The results presented in Chapter 6 provide the basis for the optimisation of biogas production from percolating packed bed digesters when linked in series. The results showed that both gas yield and the rate of solids digestion were improved using semi-continuous operation, that the operation was stable and that there was no requirement for inoculation when fresh digesters were added. It was thus necessary to optimise biogas production using this information. Hence the digester retention time was altered to determine its effect on biogas production rates. This is of great importance, as the maximum gas yields and solids losses are required with the minimum time of digester operation providing unstable conditions are not encountered.

Also of interest in this series of experiments was to establish whether the linking of digesters in series over relatively long periods would lead to the accumulation of toxic or inhibitory levels of for example ammoniacal nitrogen, by such compounds being retained within the system. In addition it was necessary to examine the extended operation of more than two units to determine there were no limiting operating factors or operational instability.

7.2.1 Digester Operating Conditions

The basic operation of digesters was as previously described in Chapter 6. A maximum of three digesters were linked in series at any one time, and in all cases a fourth digester was added at the same time as the initial digester was removed so that 'steady state' conditions were maintained. For the three unit train of digesters it can be seen that when operating at steady state a new digester is linked into the system every $^{1}/_{3}$ retention time (Fig. 7.1) and the oldest unit removed. This is refered to as the cycle time. At this point the replaced old unit will have completed one retention time. The experiment was terminated when the newest unit had completed one cycle time. The retention times used were 90, 60 and 30 days corresponding to cycle times of 30, 20 and 10 days. The experiments were thus conducted for total periods of 120, 80 and 40 days respectively. All experiments were conducted in duplicate corresponding to a total experimental time of 480 days.

The operating conditions used were a temperature of 30°C, a recirculation rate of 3 litres.hr^{-1} and a solid:liquid ratio of 2:1 with a solids loading of 6kg corresponding to a bed height of 0.51 metres.

7.2.2 Digester Monitoring

The solid substrate composition was monitored before (Table 7.1) and after digestion, a single batch of substrate being used in all the following experiments.

The liquor concentrations of volatile fatty acids, pH, total and volatile solids, total and ammoniacal nitrogen and adenosine 5' triphosphate were determined twice weekly in all experiments. Alkalinity was determined on average once per week. These assays were conducted on all digesters hence up to 45 individual assays could be conducted on the liquor phase per digester per week. All assays were conducted in duplicate and the results are presented here as averages.

Table 7.1 Solid Substrate Composition

Parameter	Mean Value	Standard Deviation
Total Solids (%)	22.84	1.69
Volatile Solids (% of T.S.)	83.99	2.23
Total Nitrogen (ppm)	5865	307
Ammoniacal - N (ppm)	1994	245
VFA (ppm)	3610	173
Cellulose (%)	13.09	0.42
Adenosine 5' triphosphate (mol/kg x 10^{-6})	0.02	0.007
non-lignin Carbon : Nitrogen Ratio 19.5 : 1		

7.3 Results and Discussion

7.3.1 Gas Production

If the results of daily gas production are examined in terms of litres of biogas per day per kilogram of solids added (Table 7.2) at each retention time, the figures quoted being the average for all digesters (D_A, D_B, D_C and D_D) operated at each retention time, major differences in the rates of gas production were noted.

It can be seen that at a retention time of 30 days (10 day cycle time), when the gas production was compared with that obtained for batch operation, over the period 0 - 10 days gas production was 0.74 $1.day^{-1}.kg^{-1}$ compared with an average of 0.87 $1.day^{-1}.kg^{-1}$ for batch operation under the same conditions, corresponding to a decrease of 14.9%. Similarly of the period 10 - 20 days gas production was 1.19 $1.day^{-1}.kg^{-1}$ compared with 1.75 $1.day^{-1}.kg^{-1}$ for batch operations, and over the period 20 - 30 days was 1.19 $1.day^{-1}.kg^{-1}$ compared with 1.39 $1.day^{-1}.kg^{-1}$ for batch operation, corresponding to reductions in gas production of 32.0% and 14.1% respectively.

At a 60 day retention time, gas production over the periods 0 - 10 and 10 - 20 days was very similar to that obtained over the same periods during batch operations. However over the period 20 - 30 days i.e. after the end of a cycle time when fresh digesters were added the gas production was found to be 2.81 $1.day^{-1}.kg^{-1}$ compared with an average of 1.39 $1.day^{-1}.kg^{-1}$ for batch operations,

Table 7.2 Average Rates of Gas Production during Semi-Continuous Operation compared to Batch Operation

Average Gas Production ($l.day^{-1}.kg^{-1}$) from all units, for the periods	30 day retention time	60 day retention time	90 day retention time	Batch Operation
0 - 10 days	0.74	0.86	0.85	0.87
10 - 20 days	1.19	1.85	1.71	1.75
20 - 30 days	1.19	2.81	1.36	1.39
30 - 40 days		1.26	1.59	1.29
40 - 50 days		1.21	0.85	
50 - 60 days		0.53	0.56	
60 - 70 days			0.72	
70 - 80 days			0.31	
80 - 90 days			0.91	

this corresponded to an increase in gas production of 102%. The rate of daily gas production then declined over the remaining 30 days. However over the period 40-50 days (after two cycle times when fresh digesters were added) the gas production was again increased (Fig.7.2).

At a retention time of 90 days gas production over the periods 0 - 10, 10 - 20 and 20 - 30 days was very similar to that obtained for batch operation. However over the period 30 - 40 days (i.e. at the end of one cycle time when fresh digesters were added) gas production was 1.59 $l.day^{-1}.kg^{-1}$ compared with an average of 1.29 $l.day^{-1}.kg^{-1}$ for batch processes. Similarly over the period 60 - 70 days (after two cycle times) gas production was again increased to 0.72 $l.day^{-1}.kg^{-1}$ when a gas production rate of 0.44 $l.day^{-1}.kg^{-1}$ might be expected during batch operation.

These results appear to show that a retention time of 60 days (cycle time 20 days) gives the optimum rate of gas production. If the rate of daily gas production at a 60 day retention time is examined more closely (Fig.7.2), it can be seen that the initial digester (D_A) attained maximum rates of gas production after approximately 12 days, a lag phase being evident due to high volatile fatty acid concentrations (Section 7.3.2). When the second digester (D_B) was added after one cycle time the gas production from D_A rapidly increased to a maximum level of 18 litres/day. In addition the lag phase of gas production from digester D_B was reduced by four days compared with batch operation (Chapter 4). This was indeed a general trend when subsequent

digesters were added. The increased gas production was due to the transfer of soluble and suspended nutrients in the liquor from the fresh digester via the recirculation system to the previous digester, this is more fully discussed in following sections. It can be seen (Fig.7.2) that at the end of two cycle times when digester D_C was added there was a large increase in gas production from digester D_B (maximum level 16 litres/day), while gas production from digester D_A was increased from 6 litres/day to 10 litres/day. This was due to the liquor from digester D_C first passing through digester D_B (causing a large increase in gas production) and then onto digester D_A. Thus the majority of nutrients passing from D_C were treated by the attached flora of D_B.

The improved rates of gas production at a 60 day retention time was in accordance with previous results (Chapters 3 and 4) which have shown that gas production together with liquor volatile solids content decreased after 20 days of operation. Thus the operation of digesters at cycle times longer than 20 days was not viable due to the low rates of gas production after this time. This was clearly shown when digester D_C was added at a 90 day retention time. The gas production from digester D_A being increased for only two days before falling back to a level of under 2 litres/day.

The low rates of gas production at cycle time 10 days were caused by nutrient overload in an unstable system and is further discuseed in Section 7.3.2.

7.3.2 Liquor Volatile Fatty Acid Concentration

At retention times of 60 and 90 days the volatile fatty acid concentration in the liquor from the initial digester (D_A) followed a pattern similar to that found for batch operation (see for example Section 4.4.1.4). If the liquor VFA concentrations are closely studied at a 60 day retention time (Fig. 7.3), it can be seen that when the second digester (D_B) was linked in the liquor VFA concentration from D_A was approximately 2100ppm. This was transfered to digester D_B, it was non-inhibitory and allowed a rapid attainment of maximum gas production, with the lag phase reduced by four days compared with batch operation. As can be seen in Figure 7.3 the VFA concentration of liquor emanating from D_B was approximately 3000ppm. This was transfered to the attached flora on the solid matrix of digester D_A where it stimulated gas production (Figure 7.2). This trend was continued in all subsequent digesters at a 60 day retention time (Table 7.3).

However in the case of a 30 day retention time (10 day cycle time) the liquor VFA concentration of digester D_A was still high (around 5000ppm) when the second digester D_B was linked (Fig.7.4). The high concentration of VFA's inhibited the start up of the second digester, leading to decreased gas production. Again this trend was continued throughout the course of the experiments and is shown in Table 7.4. If the individual volatile fatty acids are accounted for by the chromatographic method after one cycle time at both 60 and 30 day retention times (Table 7.5), i.e. when subsequent digesters were to be added, it can be seen that at a 30

Table 7.3 Average Liquor Volatile Fatty Acid Concentrations : 60 day Retention Time

Time (days)	Digester D_A	Digester D_B	Digester D_C	Digester D_D
20	2136	2940	-	-
40	2203	1961	3142	-
60	2174	2010	2307	3040
80	-	2056	2130	2289

Table 7.4 Average Liquor Volatile Fatty Acid Concentrations : 30 day Retention Time

Time (days)	Digester D_A	Digester D_B	Digester D_C	Digester D_D
10	5120	2896	-	-
20	2043	4830	3017	
30	1978	2367	4981	2831
40	-	1847	2102	5335

Table 7.5 Individual Liquor Volatile Fatty Acid Concentrations on Linking of Digesters

(Figures presented are averages)

Fatty Acid	Concentration at 30 day Retention Time (ppm)	Concentration at 60 day Retention Time (ppm)
Acetate	1176	1494
Propionate	3393	418
Iso-Butyrate	133	51
n-Butyrate	129	44
Iso-Valerate	263	72
n-Valerate	114	59
Iso-Caproate	26	14
n-Caproate	19	18
Total	5253	2107

day retention time not only was the VFA concentration high (Fig.7.4) but also that it was composed of 64% propionic acid, which is a good indicator of digester instability (Mosey, 1983). Thus the addition of further digesters at this stage would only serve to increase instability in this digester, further depressing gas production. It would also reduce the efficiency of start-up in the subsequent digesters due to the high influent VFA levels.

The individual liquor volatile fatty acid concentrations after one cycle time at a 60 day retention time are shown in Table 7.5 . Propionic acid was found to be only 19% of the total VFA's present. This indicated more stable operation, and that subsequent digesters could be linked without destabilising the system.

The results of liquor volatile fatty acid analysis have confirmed the results of gas production analysis and shown that a retention time of 30 days was too short primarily due to the instability caused by high concentrations of propionic acid.

Liquor volatile fatty acid concentrations were very similar at the longer retention times. However there were slightly higher average concentrations present at a 60 day retention time with the lower steady levels of VFA's being present for a proportionately shorter period. This was possibly the cause of more efficient gas production at this retention time, as the VFA's present were at stimulatory levels for longer periods.

Volatile fatty acid levels in the solid phase were

approximately 2000ppm at retention times of both 60 and 90 days once steady state liquor concentrations had developed. At a 30 day retention time however a trend as indicated by the liquor concentration was noted. After a full retention time liquor VFA's had decreased to 1987 (±186) ppm, the concentration in the solid phase being 2125 (±203)ppm. After two cycle times liquor VFA levels had decreased to 2317 (±221)ppm, the concentration in the solid phase being 2407 (±179)ppm. However after one cycle time liquor VFA's were at a high level of 5253 (±283)ppm which was reflected in an inhibitory concentration of 5430ppm in the solid phase. It can thus be seen that digestion was unstable in the previous solid matrix when subsequent digesters were added at a 30 day retention time.

7.3.3 Variation in Liquor pH

Liquor pH followed the trends previously described (see for example Section 6.4.3.2), being roughly the inverse of the relative liquor volatile fatty acid concentration.

The initial digesters at retention times of 90 and 60 days showed an initial decrease in pH to between 7.15 and 7.21 after eight days of operation. The pH then increased and after approximately 14 days and throughout the remainder of the experiments remained steady at 7.62 (±0.16). When subsequent digesters were added, their liquor pH was initially depressed, but to only 7.41 (±0.09) and after five days the pH throughout the recirculation system had equilibrated to approximately 7.6 units.

No significant differences in pH were noted for retention times of 60 and 90 days with all digesters having steady pH values of 7.62 (±0.16), except when minor deviations occurred when fresh digesters were added to the system. This indicated the process to be stable (and similar) at both retention times.

At a 30 day retention time, the liquor pH from the initial digester decreased to an average 7.04 (±0.20), and remained at this level until two cycle times had been completed. The pH then increased slowly, being 7.28 (±0.17) after a full retention time. Similar trends were observed for subsequent digesters, with digester D_B the pH dropped to 6.92 (±0.13) and remained at this level until two cycle times had been complete. In its final cycle time the pH of digester D_B increased to 7.25 (±0.03). The liquor from digester D_C decreased on linking to 6.93 (±0.11) and remained at this level throughout the remainder of the experiment. The pH of digester D_D, which was operated for only one cycle time also decreased on linking to an average value of 6.97 (±0.05).

The results obtained for liquor pH at a 30 day retention time correlated with the results of liquor pH, which lead to reduced gas production at this retention time. A minor discrepancy was however noted. When the liquor volatile fatty acid concentration had decreased to low levels during the last cycle time, the liquor pH did not increase to the level expected, being on average 7.27, approximately 0.35 units below that obtained in all other experiments when the liquor volatile fatty acid concentration was similar. It is believed that liquor alkalinity could be

responsible. At retention times of 90 and 60 days the average liquor alkalinities were 10908 and 10272 mg/L of $CaCO_3$ respectively. At a 30 day retention time however the average liquor alkalinity was only 3583 mg/L of $CaCO_3$. The system thus had a much poorer buffering capacity at the lowest retention time and thus relatively low volatile fatty acid concentrations could lead to low liquor pH. An inhibition of methanogens could therefore result (Boswell, 1947) causing the depression of gas production at this retention time.

7.3.4 Biogas Composition

In the initial digesters at retention times of 90 and 60 days maximum methane content of the biogas was obtained after 12 days of operation, being 60.38 (±1.53)% and 60.13 (±1.01)% respectively. The biogas methane content from digesters D_B, D_C and D_D reached maximum levels of an average 60.23 (±1.92)% after only five days at a 90 day retention time. At a 60 day retention time maximum biogas methane levels from digesters D_B, D_C and D_D of 60.04 (±1.63)% were obtained after six days. The marginally lower methane content at a 60 day retention time was probably caused by high volatile fatty acid concentrations (Section 7.3.2) which did however increase the overall biogas production of this retention time.

At a 30 day retention time, methane content of the biogas from the initial digester reached a maximum level of 60.19 (±0.98)% after 17 days of operation, i.e. towards the end of the second

cycle time. This corresponded to the decrease in liquor volatile fatty acid concentration. Similarly subsequent digesters D_B and D_C obtained maximum biogas methane content after between 17 and 19 days of operation, the concentration being 60.07 (±1.66)%. Methane content of the biogas from the fourth digester (D_D) was on average 47.33 (±3.48)% at the end of one cycle time when the experiment was terminated.

Thus the prolonged levels of volatile fatty acids at the shortest retention time lead to a slower attainment of maximum biogas methane content in all digesters, in addition to the lower rates of gas production (Section 7.3.1) at this retention time.

Thus not only was biogas production retarded at low retention times in the semi-continuous mode of operation, but its quality was also reduced. This was believed to be caused by instability within the system (evident as high volatile fatty acid concentrations) when further digesters were added.

At longer retention times, when the system had stabilised before further digesters were added, no significant compositional difference in biogas methane content was noted.

7.3.5 Solids Losses during Semi-Continuous Operation

The results of solids losses (Table 7.6), show clearly that at a retention time of 60 days solids losses were greater than at a retention time of 90 days over the same operational period. The solids lost at both these retention times were greater than those obtained for batch operation under the same conditions (Chapter 4). This is reflected in the rates of gas production attained for each regime of operation, with the increased solids reduction in the semi-continuous mode being the result of improved inoculation and start-up of fresh digesters. It can be seen from the results (Table 7.6) that there was little benefit in operation at retention times in excess of 60 days, due to the slow rate of solids degradation after this time.

At a 30 day retention time solids losses were low, in particular after one cycle time. This was most probably a result of the unstable operation when fresh digesters were added.

The results obtained were in agreement with those of Hobson *et al* (1981) who showed cellulose was not significantly degraded below a twenty day retention time due to ill-acclimatised bacteria. In this case though the bacteria may be acclimatised but conditions present in the digester were unsuitable for methanogenesis.

Table 7.6 Solids Losses during Semi-Continuous Operation

		Percentage Reduction		
		Total Solids	Volatile Solids	Cellulose
Batch Operation (after 40 days)		31.67	38.21	57.55
90 day Retention Time after:-	90 days	44.71	55.26	69.49
	60 days	38.52	49.89	53.21
	30 days	26.47	29.72	38.57
60 day Retention Time after:-	60 days	41.38	50.10	60.37
	40 days	35.16	41.37	53.82
	20 days	24.77	28.35	35.66
30 day Retention Time after:-	30 days	21.16	28.31	32.05
	20 days	14.88	22.09	24.57
	10 days	7.52	10.29	10.51

7.3.6 Effect of Semi-Continuous Operation on the Solid and Liquor Phase Nitrogen Concentrations

The liquor nitrogen concentrations were found to increase during the course of digestion in all digesters. At the end of each digestion period, irrespective of retention time or operating time, the total and ammoniacal nitrogen concentrations were found to be very similar, being on average 1917 (±209)ppm and 1371 (±243)ppm respectively. This corresponded to increases of approximately 1000ppm of ammoniacal nitrogen and 1050ppm of total nitrogen in the liquor compared with the initial inoculum. There was therefore an increase in liquor phase non-ammoniacal nitrogen of only 50ppm on average, and was probably accounted for by an increase in lignin masked nitrogenous compounds in the liquor. This would seem to indicate that liquor nitrogen concentration had little effect on the digestion process.

Ammoniacal nitrogen did not reach inhibitory levels, (van Velsen, 1977), this was particularly important at the longer retention times used.

The results obtained (Fig.7.5) show that in all cases, the liquor from freshly added digesters rapidly increased in liquor nitrogen content, this was followed by a period showing a small steady increase in nitrogen content similar to that found in the initial digester. The first stage was caused primarily by the rapid loss of transiently attached ammoniacal nitrogen from the solid matrix, occuring almost immediately upon start-up as

described in Chapter 6. This increase was found to be of between 807 and 851 ppm. The remaining increase was accounted for by the loss of non-ammoniacal nitrogen which occured more slowly in a progressive manner.

After 30 days of operation the non-ammoniacal nitrogen loss from the solid phase was found to be 324 (±72)ppm on average. This figure had increased to 543 (±91)ppm after 60 days of operation, and after 90 days of operation had increased to 725 (±166)ppm. These losses are in part accounted for by the increased liquor nitrogen concentrations, the remainder by loss from the system by one of the methods previously described.

Thus after 60 days of operation approximately 300ppm of nitrogenous matter was lost from the system, corresponding to a loss of an 5.1% of the original nitrogen input to the digester. Semi-continuous digestion over long periods therefore appeared to have little effect on the fertilising potential of the waste.

7.3.7 Liquor Total and Volatile Solids Content

At all retention times studied the liquor total solids content from all digesters was found to be similar. A mean value of 2.29% was obtained with a standard deviation of 0.26%, when fresh digesters were added their liquor dry matter content equilibrated to a similar content after only three days of operation.

The liquor volatile solids content from the initial digester (D_A) at a 90 day retention time was found to decrease from approximately 72% of the dry matter content to 38.8% after 30 days of operation. When digester D_B was added this was found to increase from 38.8% to 52.1% on average. This was due to soluble and suspended material being washed from the solid matrix of D_B, and which lead to the increase rate of gas production in D_A when D_B was linked. The volatile solids content of the liquor from D_A then decreased throughout the next cycle time and again reached a level of approximately 39%. When the third digester D_C was added, the liquor volatile solds content from D_A again increased but in this case to only 44.7% on average, from this point it decreased throughout the remainder of its retention time. The smaller increase was caused because the liquor from the fresh digester D_C was first transfered to D_B and then onto D_A. This was reflected in the increased rates of gas production from D_B and D_A which in the former were substantially greater. Similar trends were portrayed in all digesters and are shown in Figure 7.6.

At a 60 day retention time the liquor volatile solids content followed the same pattern. However, the content decreased through each retention time for each digester to a slightly higher value of on average 42.8%. This higher figure was also shown in the results of volatile fatty acid analysis (Section 7.3.2.1) where the lower steady level was found to be higher at a 60 day retention time.

Liquor volatile solids concentrations at a 30 day retention

time (Fig.7.7) were found to remain high until two cycle times had been completed for each digester, when the concentration then decreased over the final cycle time to on average 48.4% of the liquor dry matter content. This was found to be the case for each digester added and was in close agreement with the high levels of volatile fatty acids in the liquor until two cycle times had been completed.

The results obtained here confirmed that the digestion process was similar at 90 and 60 day retention times, with the latter showing an improved performance due to increased (but not toxic) levels of biodegradable material, notably volatile fatty acids. Although the results might indicate that the high levels of biodegradable material present in the liquor at a 30 day retention time should have led to increased rates of gas production, the concentration of volatile fatty acids was inhibitory and thus retarded the digestion process.

7.3.8 Adenosine tri-phosphate concentrations in the Liquid and Solid phases of Semi-continuous Digesters

Inoculum levels of adenosine 5' triphosphate were on average 3.8×10^{-6} M, and in the initial solid phase were 0.02×10^{-6} moles/kg wet weight. The liquor concentration then decreased to an average steady concentration of $1.60 (\pm 0.27) \times 10^{-6}$ M at both 60 and 90 day retention times. This concentration was maintained throughout the digestion period. When fresh digesters were added their ATP concentration in the liquor was low (0.05×10^{-6} M) as

only water and no external inoculum was added. However a concentration closely similar to that of the previous digester was rapidly attained.

This would seem to indicate that the solid matrix had a maximum bacterial population. When fresh digesters were added the nutrients transfered to the previous digester causes an increase in bacterial population within the solid matrix, which the matrix could not sustain. These additional bacteria were then transfered into the liquor and thus onto the fresh bed where they became attached and thus led to its inoculation. The solid phase population of the fresh digester then rapidly increased and an equilibrium was developed throughout the whole recirculation system. This theory of a rapidly attained and approximately finite solid matrix population when the semi-continuous mode of operation was used, was also indicated by the ATP concentrations of the matrix after various digestion times (Table 7.7). This may be as a result of the rapid transfer of a well adapted bacterial inoculum, and that there was a limit to the bacterial population the matrix could support due to factors such as nutrient availability and free attachment sites. A dynamic situation with bacterial growth and attachment rates being equal to the rates of decay and detachment will probably also enhance this effect.

At a 30 day retention time the ATP concentration within the solid phase had not increased to its maximum level further digesters were added (Table 7.7)it being only 5.44 (±0.39) x 10^{-6}moles/kg. Thus when further digesters were added the liquor

ATP concentration from the first digester dropped to approximately 0.37×10^{-6} M. This was therefore a contributory factor to the poor performance of semi-continous digesters operating at a 30 day retention time.

Table 7.7 Solid Phase Adenosine 5' triphosphate Concentrations

Period of Operation	ATP Concentration (moles/kg x 10^{-6})
10 days	5.44 (±0.39)
20 days	8.99 (±0.23)
30 days	9.82 (±0.81)
40 days	10.91 (±0.85)
60 days	9.67 (±0.37)
90 days	10.36 (±0.64)

7.4 Conclusions

1) Operation of percolating packed bed digesters in series was confirmed to be stable, with up to three digesters operating together.

2) Gas production and solids losses were improved over batch processes at retention times of 90 and 60 days, with a retention time of 60 days being optimum. Gas production was increased in the previous digester when fresh digesters were added due to the transfer of soluble or suspended nutrients via the recirculation system.

3) No external inoculum was required at any time, and inoculation by bacteria in the recirculation system was very rapid.

4) A retention time of 30 days (cycle time 10 days) was found to be unsatisfactory. The system was not stable, and this was reflected in high concentrations of volatile fatty acids, low pH, alkalinity and ATP concentration when fresh digesters are added.

5) A retention time of 60 days with a maximum of three digesters was found to be optimum, as operation needed to be stable when fresh digesters were added i.e. after around 15 days of operation. It was also found that operation over 60 days the rate of gas production was low and therefore not economic.

6) A build up of toxicants was not apparent with semi-continuous operation over long time periods, ammoniacal nitrogen concentration never approached inhibitory levels.

7) Semi-continuous operation did not significantly increase nitrogen loss from the system, it being only 5.1% over a 60 day period. Fertilising value of the waste is therefore not significantly diminished.

8) High levels of propionic acid were evident during start-up of initial digesters being present for a period of 12 days. This is a good indication of digester instabiltiy, and therefore the addition of fresh digesters at this time only leads to a compounding of these problems.

9) Methane content of the biogas was in all cases approximately 60% during stable operation and is therefore similar to batch operation under the same conditions.

10) When the cumulative daily gas production of Digesters D_B, D_C and D_D (i.e. where operation has reached a stage where it is stable) were analysed at a 60 day retention time. It can be seen (Fig.7.8) that daily gas production was between 16 and 30 litres.day^{-1}. There was thus less variation in daily gas production than for batch processes.

Figure 7.1 Start up and Operation of Digesters in a Semi Continuous Mode

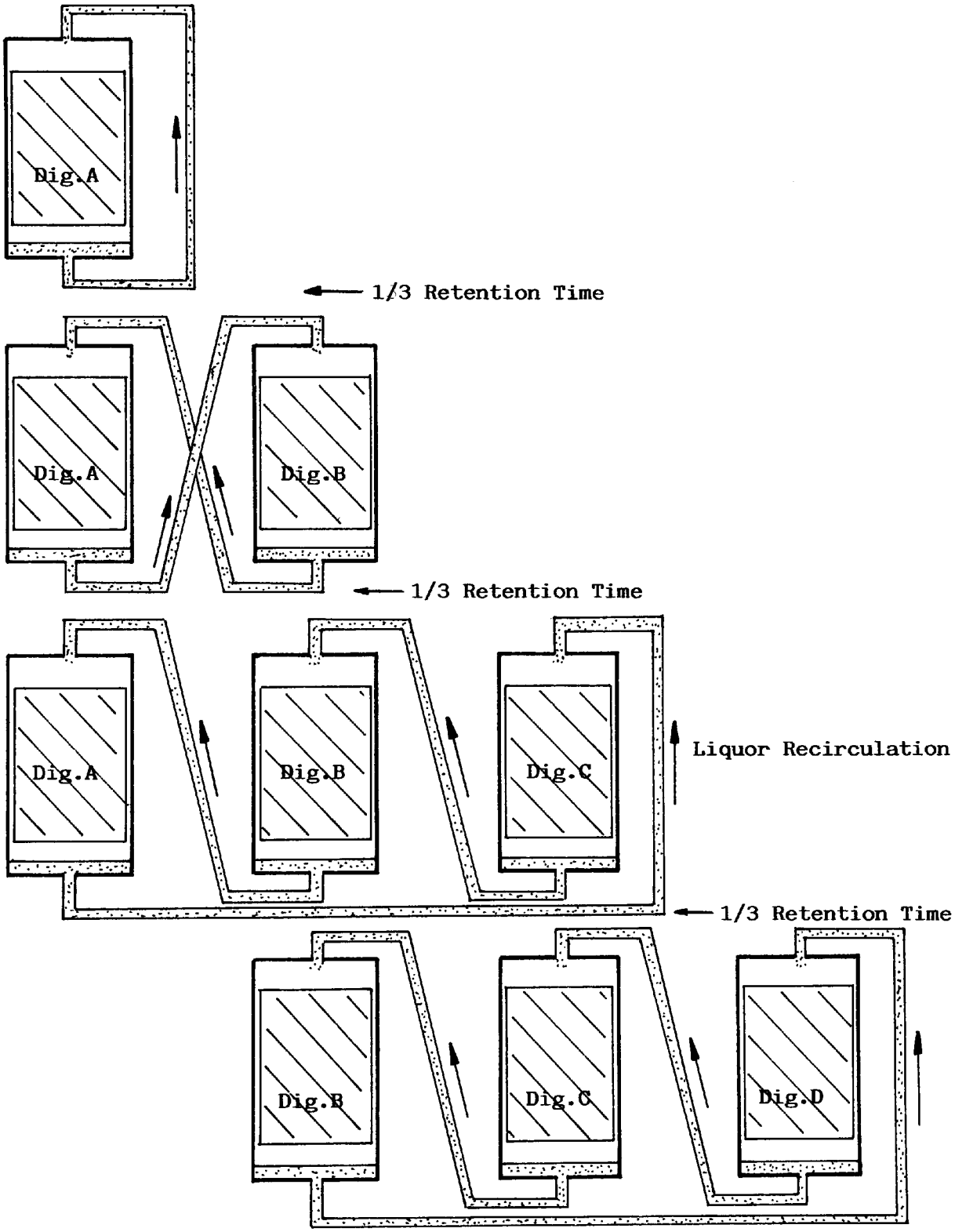


Figure 7.2 Daily Gas Production from Semi Continuously Operated Digesters at a 60 day Retention Time

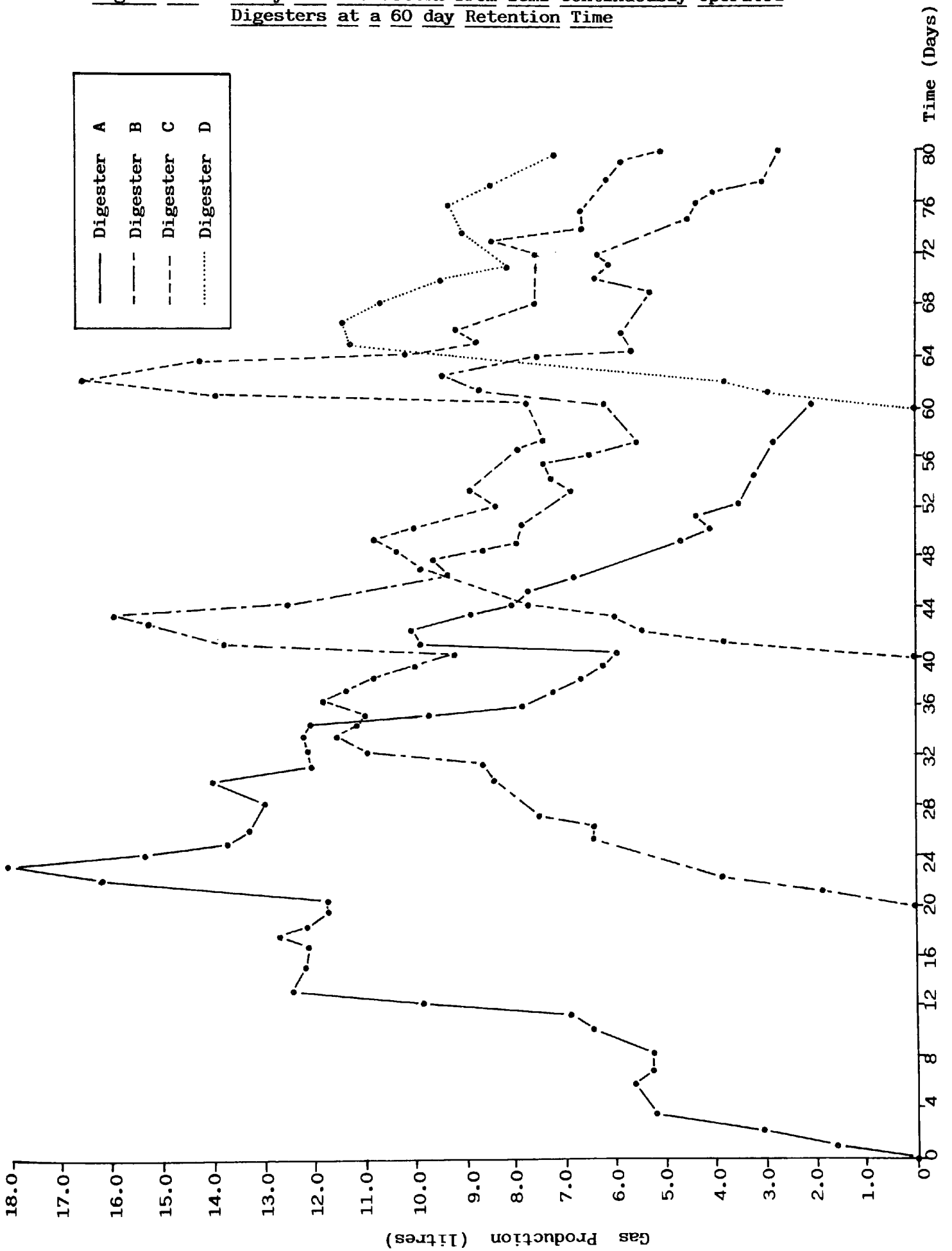


Figure 7.3 Variation in Liquor Volatile Fatty Acid Concentration : 60 day Retention Time

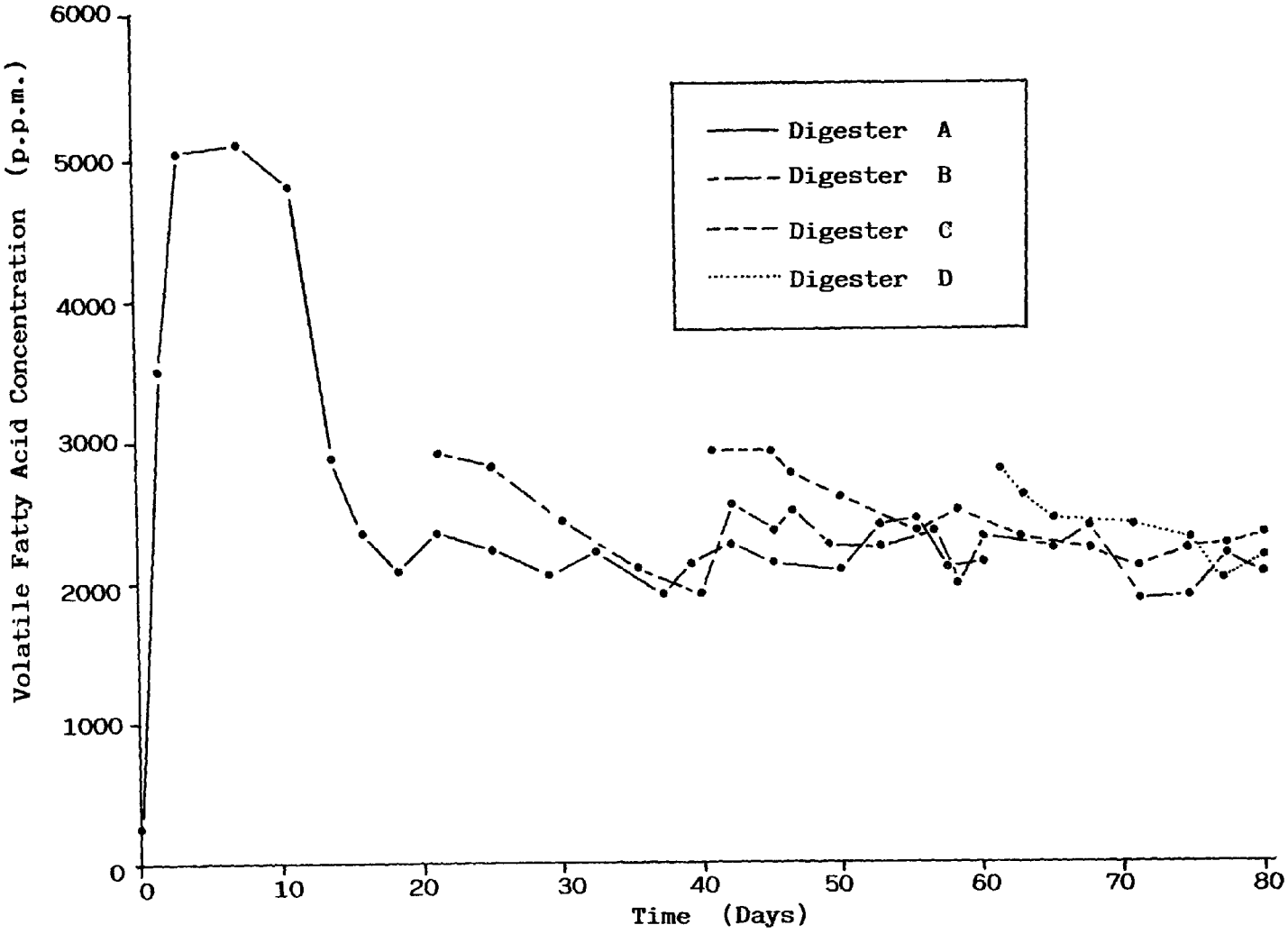


Figure 7.4 Variation in Liquor Volatile Fatty Acid Concentration ; 30 day Retention Time

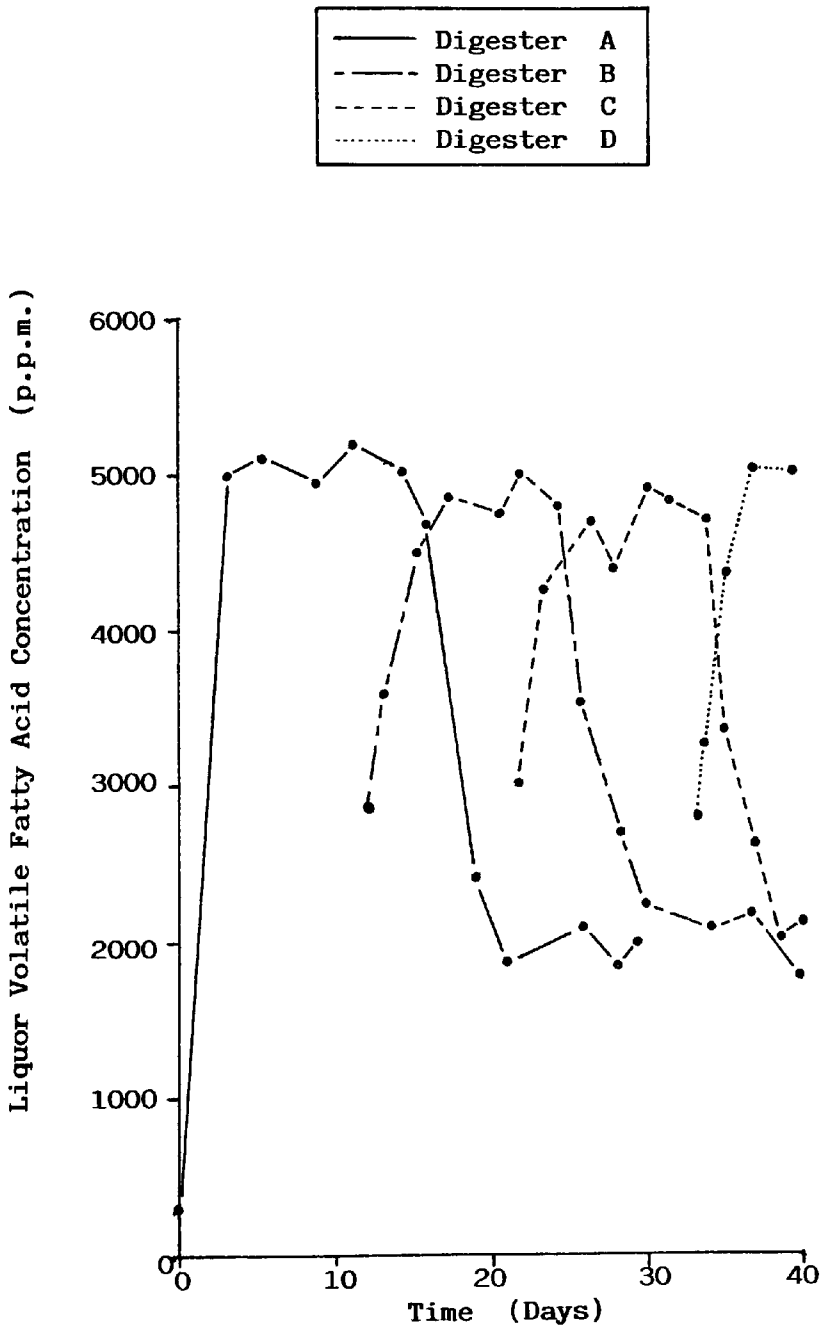


Figure 7.5 Increase in Liquor Total Nitrogen Levels : 60 day Retention Time

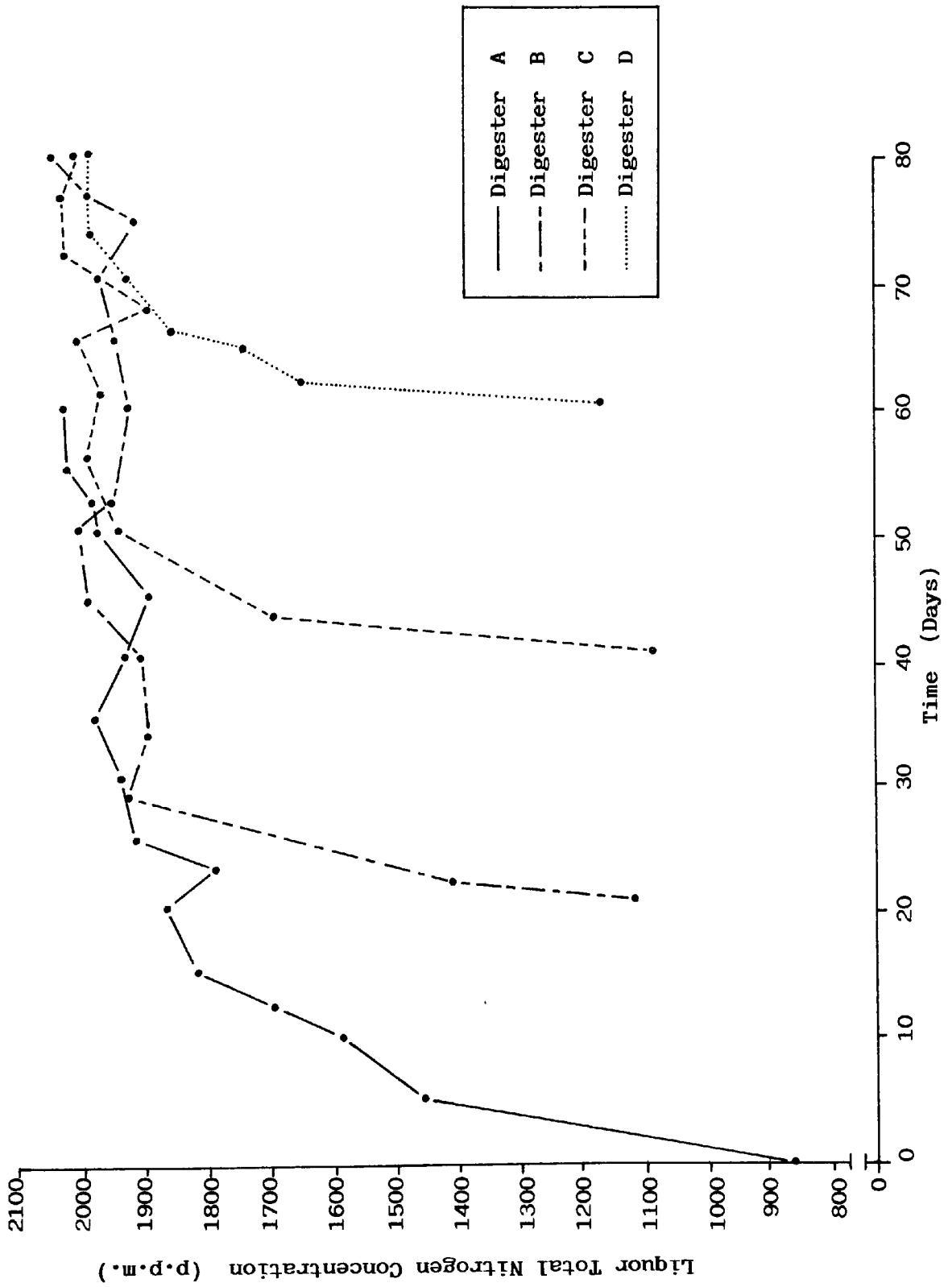


Figure 7.6 Liquor Volatile Solids Levels : 90 day Retention Time

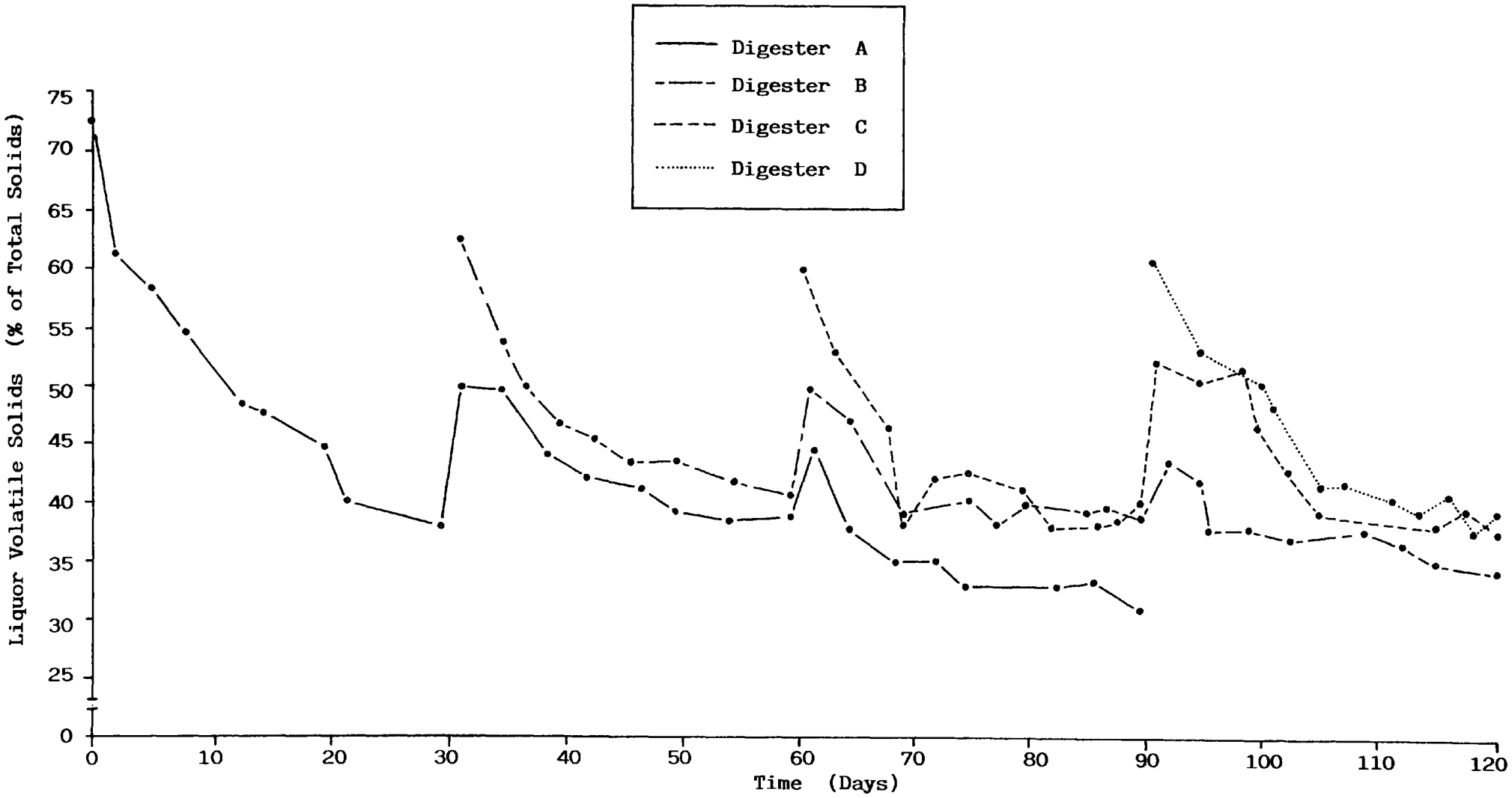


Figure 7.7 Liquor Volatile Solids Levels : 30 day Retention Time

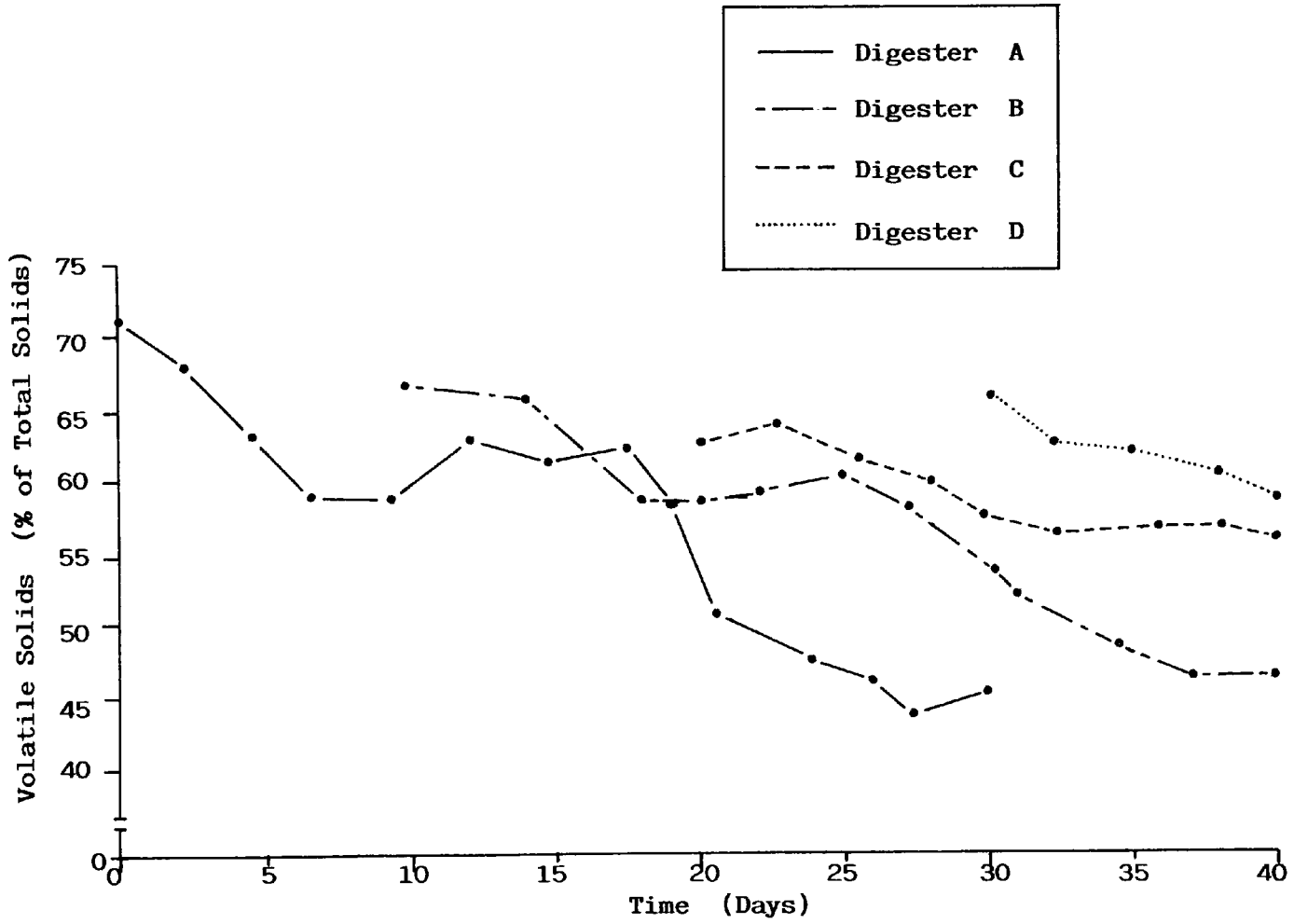
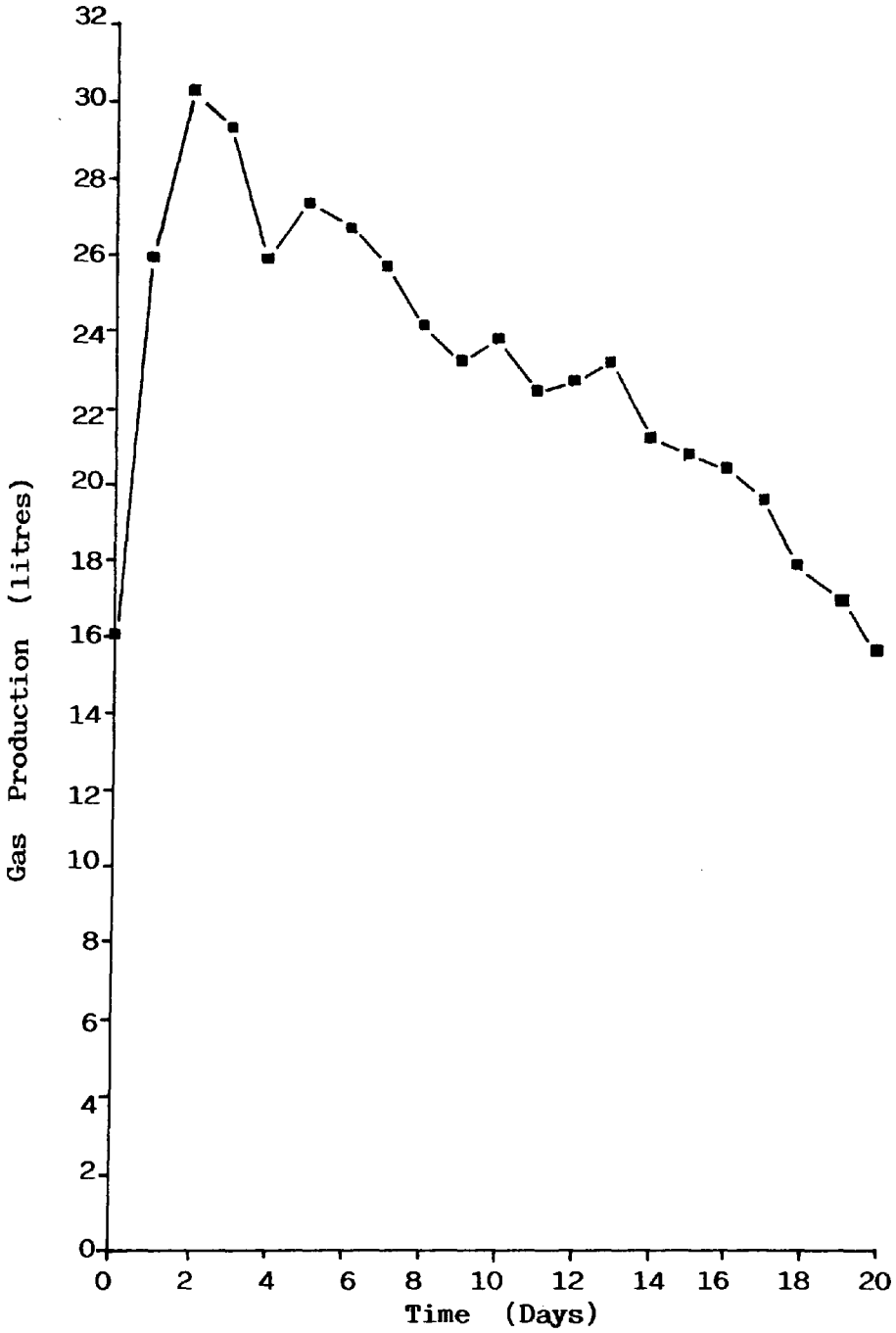


Figure 7.8 Combined Daily Gas Production

The results shown was for digesters operating at steady state at a 60 day retention time (days 60-80)



CHAPTER 8

GENERAL CONCLUSIONS

The results obtained and the operational strategies tested in the previous chapters show that the anaerobic digestion of high solids waste using this novel digester design is feasible. Its efficiency in terms of gas production and solids reduction was good and the results obtained provide a firm basis for future work. The results and conclusions of these studies are now summarised and areas requiring further work are examined.

8.1 Digester operation in a Batch Mode

The studies have shown that the operation of digesters was stable and variations in the substrate composition resulted in only minor differences in the gas yield and solids losses obtained. In all cases an increase in volatile fatty acids concentration was noted during start-up, of which around 65% of the total was propionic acid. This is generally accepted as a good indication of digester instability, and led to an inhibition of gas production during the early stages of digestion. However the system was found to be well buffered, and the high concentrations of volatile fatty acids rapidly diminished to a low steady concentration and failure (souring) of digesters did not occur.

Toxicity due to high concentrations of ammoniacal nitrogen was not encountered in this series of experiments using a cattle manure-straw substrate though problems of this nature might be

expected using for example poultry wastes.

The system was found to recover rapidly after an influx of oxygen, this was unexpected though highly beneficial, as essential maintenance can be conducted without significantly affecting the digestion process.

Gas yields of up to 0.305 m^3/kgVS added (0.673m^3/kgVS destroyed) were obtained (Fig.4.1) which compares favourably with the results of other workers, indicating that this method of digestion can produce significant amounts of biogas and provide considerable energy savings when used on farms. The biogas produced contained maximum levels of approximately 60% methane in all cases, and the time taken to reach this level was dependent mainly on the temperature of operation and the liquor volatile fatty acid concentration (Section 4.4.1.4)

Liquor VFA's were shown to play an important role in the rate of gas production, being inhibitory at concentrations of approximately 5000ppm. Once stable operation had been attained however, higher non-inhibitory VFA concentrations led to increased gas production (Section 4.4.3.3; Stafford, 1982)

Solids losses from batch digesters were high with up to 48% of the volatile solids and 65% of the cellulose content degraded over a 40 day digestion period (Table 4.4). These results are comparable with those obtained by other workers using similar wastes. Such solids losses led to a volume reduction in the order of 30% which will allow considerable space savings if the material is to be stored after digestion. The cellulose losses found could be increased by first pretreating the straw with for example sodium hydroxide, when an increase in digestibility of around 17% may be

expected (Lindberg et al, 1984), although O'Kelly (1983) found this form of chemical pretreatment to increase cellulose conversion by only 10%.

Careful evaluation of the costs involved in this type of pretreatment would have to be undertaken in order to ascertain its economic feasibility. A more practical method of pretreatment for use on farms, where cost effectiveness and simplicity of operation are of prime importance could be the use of mechanically chopped straw. Hills and Nakano (1984) have shown that gas production and volatile solids losses were increased as the particle size of tomato solids was decreased. A reduction in particle size from 20mm to 1.3mm leading to an increase in volatile solids losses from 21% to 60%.

The experiments showed that gas production was highest at 35°C, the other temperatures investigated being 30°C and 25°C. However, increased gas production at higher temperatures may be offset by the heat input required to maintain digester temperature. The greater operating temperature is above ambient temperature, the more biogas will be required to maintain digester temperature. It is possible therefore that a temperature lower than 35°C will be optimal for net energy production in a full scale unit (Hawkes and Horton, 1981)

It may be argued however that percolating packed bed digesters have a higher optimum temperature of operation than conventional digesters. High solids digesters will have a smaller surface area for heat loss to occur, and thus maintainence of temperature will require lower heat input. In addition the substrate used will have a smaller volume and lower water content

and will thus require a lower heat input to attain digester operating temperature.

As this type of waste would most probably be used as a soil conditioner and fertiliser, it was important that its nitrogen content was not significantly affected by digestion. It was found that only 3-4% of the initial nitrogen content was lost from the system, probably as ammonia vapour. Its potential as a fertiliser was therefore not significantly affected.

The results showed that solid:liquid ratio was highly important (Section 4.4.3), with a ratio of 2:1 being optimal, mainly as a result of increased liquor metabolite concentration.

Increased bed height caused an increased gas yield up to a critical point when compaction followed by blocking of the bed occured. The critical height was found to be approximately 1.5 metres at a digester diameter of 0.18 metres using a straw-manure waste of 27% total solids. Providing blockages did not occur efficiency was increased with bed height due primarily to increased liquor residence times (Section 4.4.4.3).

Experiments into the effect of liquor recirculation rate indicated that the bacteria present are tenaciously attached to the solid matrix, as gas yield was not greatly affected even at a recirculation rate of 15 litres.hr^{-1} (Fig.4.5). This may be a result of the surface roughness of the straw fibres due to the action of cellulases.

Low temperatures appeared to adversely affect the growth and metabolic rates of methanogens more than non-methanogens. This could have important implications on digester start-up. The use of higher temperatures at start up could lead to stable conditions

being more rapidly attained. It is possible that the operating temperature could then be reduced, thus less heating would be required to maintain digester temperature.

The analysis of particle size distribution showed that after 10 days of operation only 5% of the liquor dry matter was above 75μm in size, and that the cellulose content of the liquor is reduced by 95% in 40 days of operation. The solid matrix thus appears to act as a filter. This has important implications in the avoidance of blockages in the recirculation system caused by the accumulation of fibrous particles.

Evidence was found for a highly degradable sulphur containing compound to be present in the waste. Hydrogen sulphide concentrations in the biogas increased to approximately 4000ppm (Fig.5.2) during the early stages of digestion. It was not clear if the hydrogen sulphide originates from sulphur reducing bacteria or methanogenic bacteria but it is probable that the high levels of hydrogen sulphide are contributing to the inhibition of methane production in the early stages of digestion.

Many of the investigations conducted indicate a rapid colonisation of the solid matrix. The relative rates of gas production from the solid and liquid phases show that the solid phase accounts for 85% of the biogas production after 7 days of operation (Fig.5.3). Methane content of the biogas indicated that methanogens colonised the bed at a slower rate. This may indicate that methanogens require greater levels of surface irregularity before attachment occurs or that the slower attachment was a result of inhibition caused by high volatile fatty acid concentrations and high hydrogen sulphide concentrations reducing their growth rate.

This indeed appeared to be shown by semi-continuous operation, when the methane content of the biogas from fresh digesters increased very rapidly and where VFA concentrations are not inhibitory (Section 6.4.1). Analysis of the adenosine 5' triphosphate (ATP) concentrations also indicated a rapid colonisation of the solid matrix, with the concentration rising rapidly to a maximum of 10×10^{-6} moles.kg^{-1} (Section 5.3.4). After 20 days of operation the liquor and solid phase ATP concentrations are steady, indicating a dynamic bacterial population, with the rates of attachment and growth being roughly equal to detachment and decay. These results were confirmed by microscopic evaluation, which showed a rapid colonisation particularly to areas of the bed affected by the action of cellulases. Viable counts of the liquor bacteria after 20 days of operation (Section 5.3.6) showed $10^6/10^7$ methanogens.ml^{-1} and $10^7/10^8$ non-methanogens.ml^{-1} to be present, in close agreement with the results of other workers.

In conclusion these results have shown the principle of percolating packed bed digestion to be sound, and that biogas produced could positively affect the economics of many small farms.

8.2 Digester Operation in a Semi-Continuous Mode

Studies pertaining to the semi-continuous operation of percolating packed bed digesters (Chapter 6), showed that the operation of the system in this manner was not only feasible, and would allow the treatment of waste produced continuously, but would also increase the efficiency of the process.

Initial studies showed that the gas production from

semi-continuous operation was increased by 18% over that obtained from the operation of batch digesters over the same time period (Section 6.4.1). The increased gas production was due to the rapid transfer and therefore colonisation of well adapted bacteria to the fresh digester via the recirculation system. In addition, soluble and suspended metabolites were transfered in the recirculation stream from the fresh digester and were treated by the attached flora of the initial digester thus leading to an increase in biogas production. There was therefore no build up of volatile fatty acids when fresh digesterswere added, and thus no inhibition occured. Because of the well adapted bacteria present in the recirculation stream, no new inoculum was required to initiate digestion in subsequent reactors. These factors resulted in increased solids losses in semi-continuous digesters of approximately 20% compared with batch processes (Section 6.4.4). The results showed a significant fall in gas production after around 20 days of operation and that after 60 days the rate of daily gas production was very low and operation beyond this time was economically unsound. The operation of a maximum of three digesters in series confirmed these results and showed a retention time of 60 days to be optimum. This corresponds to a 20 day cycle time. At cycle times of 15 days and below, high volatile fatty acid concentrations were still prevalent, with propionate accounting for approximately 65% of the total volatile fatty acids which suggests digester operation was unstable (Section 7.3.2). At retention times of over 60 days, the gas production was only marginally increased, as were the solids losses, most probably as a result of the poor biodegradability of the waste remaining after

this time.

Semi-continuous digestion did not adversely affect the preservation of nitrogenous components, and hence its fertilising potential. The linking of digesters did not cause an increase in ammoniacal nitrogen levels, thus inhibition due to ammonia was not encountered. In addition, there was no apparent increase in any toxic components when digesters were operated over long periods (up to 120 days) in a semi-continuous mode.

The system was found to be well buffered throughout, and the pH to be in excess of 7.5 except in the case of a 30 day retention time (Fig.7.3) when fresh digesters were added before the previous one has stabilised, though even in this case souring of digesters did not occur.

The ATP concentration within the solid matrices of fresh digesters was found to increase very rapidly, probably as a result of a well adapted inoculum being transfered.

Thus the operation of percolating packed bed digesters in series was found to be stable (above a retention time of 30 days) and gave increased gas production and solids losses when compared with batch operations over the same time scale. In addition to this, when three digesters are operating in steady state it was found that their combined daily gas production was more regular than for similar batch operations. This is important, as the minimum daily gas production dictates the volume of utilisible biogas which is known to be continuously available for heating or electricity generation.

8.3 Further Work

The results presented here provide a firm basis for further study of the system at a scale closer to that which might be used to treat straw-manure substrates on a farm situation, or for the treatment of other solid wastes.

A number of studies are still required before investment in pilot scale equipment is made. For example the semi-continuous operation of laboratory scale digesters over periods of perhaps a year in order to determine that no gradual build-up of toxicants occurs. It will also be important to examine the effects of intermittent liquor recirculation at a laboratory scale, the results to date indicate that this would be possible, and pumping perhaps 15 minutes per hour once stable operation was established could lead to considerable energy savings.

Examination of the digestion of other wastes using percolating packed bed digesters is also required, and some possible substrates are grass clippings, municiple solid waste, poultry wastes and solid vegetable wastes such as peelings and tops. It is possible that the optima found for manure-straw mixtures will not be pertinent here and additionally problems not encountered such as ammonia toxicity (especially with poultry wastes; see Webb, 1984), poorly buffered systems and the requirements of an additional nitrogen source (for wastes with very high carbon : nitrogen ratios) may be found and the digestion strategy altered to accommodate these factors.

The operational parameters and digestion efficiencies obtained for different wastes will be invaluable in determining the

best waste with which to evaluate pilot scale operation of the system.

A further option with this type of system, particularly for wastes not produced on a year round basis, is to use the solid matrix of percolating packed bed digesters as a conventional anaerobic filter to treat low solids liquid waste. For example, in the case of dairy farms, the digester could be used as described here, and once the waste was exhausted, the attached bacteria could be used for the treatment of parlour washings. Thus the time period bacteria remain viable in a spent reactor needs to be evaluated.

The full scale system envisaged from this work would most probably involve steel or reinforced concrete digesters sunk in the ground. The material to be digested would be loaded into perforated metal baskets which would be lowered into the digesters from an overhead gantry. To overcome the problems of compaction elucidated here, and which are likely to be worsened at increased digester diameters, it will probably be necessary to have digesters which are vertically compartmented in order to benefit from increased liquor residence times while avoiding compaction. This could be accomplished by loading the waste into a number of baskets which would be loaded vertically in the digester. These digestion strategies will require experimentation on a pilot scale reactor to elucidate if they are viable before full scale trials can be considered.

REFERENCES

Ait, N., Creuzet, N., Forget, P.
Partial purification of cellulose from Clostridium thermocellum
J. Gen. Micro., 113, 399, 1979.

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C.
Chemical analysis of ecological materials
(Allen, S.E., ed.) Blackwell Scientific, Oxford, 1974.

Anon.
Methane gas as a vehicle fuel
Wat. Res. News, 10, 4, 1981.

Archer, D.B.
The Microbial basis of process control in methanogenic fermentation of Soluble Wastes
Enzyme Microb. Technol., 5, 162, 1983.

Ashare, E., Wentworth, R.L., Wise, D.L.
Fuel gas production from animal residues
Resour. Recovery. Conserv., 3, 343, 1979

Baccay, R.A., Hashimoto, A.G.
Acidogenic and methanogenic fermentation of cauticized straw
Biotech. Bieng., 26, 885, 1984

Balch, W.E., Magrum, L.J., Fox, G., Wolfe, R.S., Woese, C.R.
An ancient divergence amongst the bacteria
J. Mol. Evol., 9, 305, 1977

Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R., Wolfe, R.S.
Methanogens : Reevaluation of a unique biological group
Microbiol. Rev., 43(2), 260, 1979

Balch, W.E., Wolfe, R.S.
New approach to the cultivation of methanogenic bacteria
Appl. Environ. Microbiol., 32(6), 781, 1976

Van den Berg, L.
Effect of temperature on growth and activity of a methanogenic culture utilising acetate
Can. J. Microbiol., 23(7), 898, 1977

Van den Berg, L.
Anaerobic digestion of wastes
Conservation and Recycling, 5(1), 5, 1982

Van den Berg, L., Kenndey, K.J.
Performance characteristics of anaerobic downflow stationary fixed film reactors
Proc. 1st. Intl. Symp. on Fixed Film Processes, Kings Island, Apr. 1982.

Van den Berg, L., Kenndey, K.J.
Comparison of advanced anaerobic reactors
Proc. 3rd. Intl. Symp. on Anaerobic Digestion,
Aug. 1983, Boston, Mass., pp.71-89, 1983

Binto, R.A., Navaeu, A.P., Nyns, E.J.
Methanogenic potential of mixed liquors; fluorimetric monitoring
Biotech. Letts., 3(11), 623, 1981

Bligh, E.G., Dyer, W.J.
Can. J. Biochem. Physiol., 37, 911, 1959

Boone, D.R., Bryant, M.P.
Propionate degarding bacterium Sytrophobacter wolinii sp. nov. gen. nov., from methanogenic ecosystems
Appl. Environ. Microbiol., 40, 626, 2980

Bousfield, S., Hobson, P.N., Summer, R.
A note on the anaerobic digestion of poultry waste
Agric. Wastes, 1, 161, 1979

Box, E.
Primary productivity of the biosphere
(Lieth, H., Whittaker, R.H. eds.) Springer Verlag, New York, p.265, 1975

Braun, M., Schoberth, S., Gottschalk, G.
Enumeration of bacteria forming acetate from H_2 and CO_2 in anaerobic environments
Arch. Microbiol., 120, 201, 1979

Bridgewater, A.V.
Technological economics applied to waste recovery and treatment processes : capital cost estimation
Effluent and Water Treatment Jnl., 17, 337, 1977a

Bridgewater, A.V.
Technological economics applied to waste recovery and treatment processes : cost data and operating costs
Effluent and Water Treatment Jnl., 17, 467, 1977b

Bryant, M.P.
Commentary on the Hungate technique for the culture of anaerobic bacteria
Am. Jnl. Clinical Nutrition, 25, 1324, 1972

Bryant, M.P., Burkey, L.A.
Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen
J. Dairy Sci., 36, 205, 1953

Bryant, MP., Wolin, E.A., Wolin, M.J., Wolfe, R.S.
Methanobacillus omelianski; a symbiotic association of two species of bacteria
Arch. Microbiol., 59, 20, 1967

Bull, M.A., Sterritt, R.M., Lester, J.N.
The distribution of bacterial activity in a fluidised bed reactor
Wat. Res. 18(8), 1917, 1984

Buswell, A.M. 1947
Important considerations in sludge digestion, Part II
Sewage Works Jnl. 19(1), 28, 1947

Callander, I.J., Barford, J.P.
Improved anaerobic digetion of pig manure through increased retention of substrate and bacterial solids
Biotech. Letts., 5(3), 147, 1983a

Callander, I.J., Barford, J.P.
Recent advances in anaerobic digester technology
Proc. Biochem., Aug. 1983, 24, 1983b

Carruthers, S.P.
Catch crops for energy
In : "Energy from Biomass" (Strubb, A. et al., eds)
Applied Science, London, p.127, 1982

Chandler, J.A., Jewell, W.J., Gosset, J.M., van Soesk, P.J., Robertson, J.B.
Predicting Methane Fermentation Biodegradability
Biotech. Bioeng. Symp. Ser., 10, 93, 1980

Chou, W.L., Speece, R.E., Siddique, R.H.
Acclimation and degradation of petrochemical wastewater components by methane fermentation
Biotech. Bioeng. Symp. Ser., 78, 391, 1978

Colberg, P.J., Young, L.Y.
Biodegradation of lignin derived molecules under anaerobic conditions
Can. J. Microbiol., 28, 886, 1982

Colleran, E.
Pers. comm. 1985 | niversity of Galway, Ireland.

Colleran, E., Booth, A., Barry, M., Wilkie, A., Newell, P.J., Dunican, L.K.
Bioconversion of agricultural wastes into fuel gas and animal food
In: Energy from Biomass (Palz, W., et al eds) Applied Science Publ., London, 1980

Colleran, E., Barry, M., Wilkie, A., Newell, P.J.
Anaerobic digestion of agricultural wastes using the upflow anaerobic filter design
Proc. Biochem., March/April 1982, 12, 1982

Das, D., Ghose, T.K., Joshi, A.P., Gopalkrishnan, K.S.
Treatment of distillery wastes by a two phase biomethanation process
Energy Biomass Wastes, 7, 601, 1983

Dimovski, C.
Anaerobic sludge digestion process with prolonged digestion time for the solid substances and shortened for the sludge liquor
Prog. Wat. Tech. 12(3), 307, 1980

Duff, S.J.B., Kennedy, K.J.
The effect of effluent recirculation on start-up and steady state operation of the downflow stationary fixed film reactor
Biotech. Letts. 5(5), 317, 1983

Ehrig, H.J.
Laboratory tests for anaerobic digestion of municipal solid waste
Poster Presentation. Third Intl. Symp. on Anaerobic Digestion, Boston, Mass., August 1983

Farooq, S., Dasgupta, A., Daly, E., Gerrish, H.P., Sengupta, S., Wong, K.F.
Prototype demonstration studies of methane production from municipal solid waste at Pompano Beach, Florida.
Altern. Energy source, 3(7), 313, 1983

Finck, J.D., Goma, G.
Anaerobic digestion of lignocellulosic material; description of a new continuous process
Proc. 2nd E.C. Conf. "Energe from Biomass", Berlin, Sept. 1983, p.1033, 1983

Floyd, J.R.S.
Operation of a tubular anaerobic digester for piggery waste
Ph.D. Thesis, CNAA, Aug. 1984

Forsberg, C.W., Lam, K.
Use of ATP as an indicator of the microbiota in rumen contents
Appl. Environ. Microbiol., 33, 528, 1977

Fox, G.E., Magrum, L.J., Balch, W.E., Walfe, R.S., Woese, C.R.
Classification of Methanogens
Proc. Natl. Acad. Sci. USA, 74, 4537, 1977

Frostell, B.
Anaerobic fluidised bed experimentation with a molasses wastewater
Proc. Biochem., 17(6), 37, 1982

Ghosh, S., Conrad, J.R., Packer, M., van Ryzin, E.
Anaerobic Processes
J.W.P.C.F., 48(6), 1115, 1976

Ghosh, S. Klass, D.L.
Two phase anaerobic digestion
Proc. Biochem., 13, 15, 1978

Ghosh, S., Sajjad, A., Henry, M.P., Bleakney, R.A.
Two stage upflow anaerobic digestion of concentrated sludge
Biotech. Bioeng., 13, 351, 1983

Goldberg, T., Bjergbok, B., Djerneas, E., Moritzen, A.
An anaerobic digester for continual high solids loading : the biofunnel
Proc. Intl. Gas Res. Conf., 1981

Groleau D., Forsberg, C.W.
Cellulolytic activity of the rumen bacteria Bacteroides succinogens
Can. J. Microbiol., 27, 517, 1981

van Gylswyk, N.O.
Fusobacterium polysaccharolyticum sp. nov., a gram negative rod from the rumen that produces butyrate and ferments cellulose and starch
J. Gen. Microbiol., 116, 157, 1980

H.M.S.O.
The analysis of raw, potable and waste water
H.M.S.O., London, 1972

H.M.S.O.
The analysis of agricultural materials
H.M.S.O., London, 1981

Hall, E.R., Jovanic, M., Pejic, M.
Pilot studies of methane production in fixed film and sludge blanket anaerobic reactors
Bioenergy Res. Dev., 4, 475, 1982

Hall, S.J., Hawkes, D.L., Hawkes, F.R., Thomas, A.
Mesophilic anaerobic digestion of high solids cattle waste in a packed bed digester
J. Agric. Engng. Res., 32, 153, 1985a

Hall, S.J., Hawkes, D.L., Thomas, A., Hawkes, F.R.
Optimisation of biogas production from a high solids waste - batch process
Proc. Adv. in Fermentation II, Chelsea College, London, 23-25 Sept. 1985b

Hanaki, K., Matsuo, T., Nagase, M.
Mechanism of inhibition caused by long chain fatty acids in anaerobic digestion processes
Biotech. Bioeng., 23(7), 1591, 1981

Harvey, M., Forsberg, C.W., Beveridge, T.J., Pos, J., Ogilvie, J.R.
Methanogenic activity and structural characteristics of the microbial biofilm on a needle punched polyester support
Appl. Environ. Microbiol. 48(3), 633, 1984

Hashimoto, A.G.
Methane production and effluent quality from fermentation of beef cattle waste
Biotech. Bioeng., 11, 481, 1981

Hashimoto, A.G.
Methane from swine wastes : Effect of temperature and influent concentration on kinetic parameter K
Agric. Wastes, 9, 299, 1984

Hashimoto, A.G., Chen Y.R.
The overall economics of anaerobic digestion
In: "Anaerobic Digestion " (Stafford, D.A. et al., eds)
Applied Science, London, p449, 1980

Hawkes, D.L.
Factors affecting net energy production from anaerobic digesters
In: "Anaerobic Digestion" (Stafford, D.A. et al., eds)
Applied Science, London, p.131, 1980

Hawkes, D.L., Horton, H.R.
Optimisation of anaerobic digesters for maximum energy production
Studies in Environ. Sci., 9, 137, 1981

Hawkes, F.R., Floyd, J.R.S., Hawkes, D.L.
Operation of laboratory scale plug flow type digesters
Proc. Ist. E.C. Conf. "Energy from Biomass", Applied Science, London, p.318, 1981

Hawkes, F.R., Young, B.V.
The design and operation of laboratory scale anaerobic digesters. Operating experience with poultry waste
Agric. Wastes, 2, 119, 1980

Hill, D.T.
A comprehensive dynamic model for animal waste methanogenesis
Trans. A.S.A.E., 25(5), 1374, 1982

Hills, D.J.
Effect of C : N ratio on anaerobic digestion
Agric. Wastes, 1(4), 267, 1979

Hills, D.J.
Methane gas production from dairy manure at high solids concentration
Trans. A.S.A.E., 23(1), 122, 1980a

Hills, D.J.
Biogas from a high solids combination of dairy manure and barley straw
Trans. A.S.A.E., 23(6), 1500, 1980b

Hills, D.J., Nakano, K.
Effects of particle size on anaerobic digestion of tomato solid wastes
Agric. Wastes, 10, 285, 1984

Hills, D.J., Roberts, D.W.
Methane gas production from dairy manure and field crop residues
Proc. 4th Intl. Symp. on Livestock Waste, p.92, 1980

Himmel, W., Lafferty, R.M.
Operating experience with a double chamber digester with cattle manure containing bedding straw as a substrate
Proc. A.W.W.T. Conf., Noordwijkerhout, Netherlands, 23-25 Nov., 1983

Hobson, P.N.
Microbiology of anaerobic treatment processes
J. Appl. Chem. Biotechnol., 24, 375, 1974

Hobson, P.N.
Anaerobic digestion of animal excreta and other agricultural waste
Trib. Cebedeau., 34, 437, 1981

Hobson, P.N.
Biogas production from agricultural wastes
Experienta, 38, 206, 1982

Hobson, P.N. Bousfield, S., Summers, R.
Methane production from agricultural and domestic waste. In: Energy from Waste Series (Porteous, A. ed.) Applied Science, London, 1981

Hobson, P.N., Shaw, B.G.
The anaerobic digestion of wastes from an intensive pig unit
Wat. Res., 7, 267, 1973

Hotchkiss, M.
Factors influencing the bacterial flora in an Imhoff Tank
J. Am. Publ. Health Assoc., 15, 702, 1952

Hungate, R.E.
Studies on Cellulose Fermentation
J. Bacteriol, 48, 499, 1954

Huysman, P., van Meenan, P., van Assche, P., Verstraete W.
Factors affecting the colonisation of non-porous and porous packing materials in model upflow methane reactors
Biotech. Lets., 5(9), 643, 1983

Jain, M.K., Singh, R., Tauro, P.
Cattle waste anaerobic digestion in a semi-continous and a batch process
Agric. Wastes, 7, 251, 1983

Jeris, J.S., McCarty, P.L.
Cation toxicity and stimulation in anaerobic waste treatment
J.W.P.C.F., 37, 97, 1965

Jewell, W.J.
Future trends in digester design. In: Anaerobic Digestion (Stafford, D.A. et al, eds.), Applied Science, London, p.467, 1980

Jewell, W.J., Dell Orto, S., Fanfoni, K.J., Hayes, T.D., Leuschner, A.P., Sherman, D.F.
Low cost methane generation on small farms
Proc. 3rd Ann. Symp. on Biomass Energy Systems, p.547, 1979

Jewell, W.J., Dell Orto, S., Fanfoni, K.J., Fast, S., Jackson, D., Kabrick, R.M.
Dry anaerobic methane fermentation
Proc. 2nd. Sem. on Biomass energy for City, Farm and Industry, p.157, 1981

Kandler, O., Hippe, H.
Lack of peptidoglycon in the cell walls of Methanosarcina barkeri
Arch. Microbiol., 113, 57, 1977

Kandler, O., König, H.
Chemical composition of the peptidoglycon free cell walls of methanogenic bacteria
Arch. Microbiol., 118, 141, 1978

Klein, A., Schnoor, M.
Genome complexity of methanogenic bacteria
J. Bacteriol., 158(2), 628, 1984

König, H., Kandler, O.
The amino acid sequence of the peptide moiety of the pseudomurein of Methanobacterium thermoautotrophicum
Arch. Microbiol., 121, 271, 1979

Koster, I.W.
Liquefaction and acidogenesis of tomatoes in an anaerobic two-phase solid waste treatment system
Agric. Wastes, 11, 241, 1984

Kottowitz, D.A., Schulte, D.D.
Tumble-mix anaerobic digestion of dry beef manure
A.S.A.E. Microfiche collection, No.82-3596, 1982

Kroiss, H., Wabnegg, F.
A testing method to characterise anaerobic sludge and anaerobic removal of substrates
Poster presentation, 3rd, Intl. Symp. on Anaerobic Digestion, Boston, Mass., August 1983

Landine, R.C., Brown, G.J., Cocci, A.A., Viraraghaven, T.
Anaerobic treatment of high strength, high solids potato waste
Agric. Waste., 7, 111, 1983

Larkin, S.B.C.
The distribution of agricultural wastes and residues in the U.K.
The Digest, 6, 3, 1982

Laura, R.D., Idnami, M.A.
Increased production of biogas from cow dung by adding other agricultural wastes
J. Sci. Fd. Agric., 22, 164, 1971

Leeper, S.A., Wolfram, J.H., Chow, M.L., Dawley, L.J.
Municiple solid waste bioconversion technologies
U.S. Dept. of Energy, Report No. DEAC07-761, 1982

Lettinga, G., Halshoff, L.W., Pol, W., Weignant, W., de Zeeuw, W., Hobima, S.W., Grin, P., Poensman, R.
Upflow anaerobic sludge processes
Proc. 3rd Intl. Symp. on Anaerobic Digestion. Boston, Mass., Aug. 1983, p.139, 1983

Lindberg, J.E., Ternrud, I.E., Theander, O.
Degradation rate and chemical composition of different types of alkali treated straw during rumen digestion
J. Sci. Food Agric., 35, 500, 1984

Mah, R.A., Sussman, C.
Microbiology of anaerobic sludge fermentation : enumeration of non-methanogenic anaerobic bacteria
Appl. Microbiol., 16(2), 358, 1967

Mah, R.A., Ward, D.M., Baresi, L., Glass, T.L.
Biogenesis of Methane
Ann. Rev. Microbiol., 31, 309, 1977

Maramba, F.D., Judan, A., Obias, E.
Fuel, feed and fertilizer from farm wastes; the Phillipine experience
Proc. 3rd Intl. Symp. on Anaerobic Digestion. Boston, Mass., Aug 1983, p357, 1983

Marchaim, U.
Anaerobic digestion of agricultural wastes; the economics lie in the effluent uses
Proc. 3rd Intl. Symp. on Anaerobic Digestion. Boston, Mass., Aug. 1983, p.343, 1983

McCarty, P.L.
Anaerobic waste treatment fundamentals, Part III
Public Wks., 95(11), 91, 1964

McCarty, P.L.
One hundred years of anaerobic digestion. In: "Anaerobic Digestion 1981" (Hughes, D.E. et al, eds.) Elsevier Biomedical, Amsterdam, p.3, 1982

McCarty, P.L., McKinney, R.E.
Salt toxicity in anaerobic digestion
J.,W.P.C.F., 33, 399, 1961

McCarty, P.L., Young, L.Y., Healy, J.B., Owen, W.F., Stuckey, D.C.
Thermochemical treatment of lignocellulosic and nitrogenous residuals for increasing anaerobic biodegradability
Proc. 2nd Ann. Fuels Biomass Symp., p787, 1978

Mills, P.J., Hobson, P.N., Bousfield, S., Summer, R.
Anaerobic digestion of farm wastes
I. Chem. E. Proc. Ann. Res. Meeting, p.1, 1979

Montgomery, H.A.L., Dymock, J.F., Thom, N.S.
The rapid colourimetric determination of organic acids and their salts in sewage sludge liquor
The Analyst, 87, 949, 1962

Mosey, F.E.
New Developments in the anaerobic treatment of industrial waste
Effl. Wat. Treatment Jnl., 85, 1983

Oakley, D.L., Forster, C.F., Wase, D.A.J.
Bacterial attachment in relation to the operation of an expanded bed reactor
Proc. Adv. in Fermentation II, Chelsea College, London, 23-25 Sept. 1985, p.20, 1985

O'Kelly, N., Barry M., Colleran, E.
Laboratory scale studies on the anaerobic digestion of agricultural residues by a two stage process
Poster Presentation, 3rd Intl. Symp. on Anaerobic Digestion, Boston, Mass., Aug. 1983

Parkin, G.F., Speece, R.E.
Attached versus suspended growth anaerobic reactors; response to toxic substances
Wat. Sci. Technol., 15, 261, 1983

Patel, G.B., Khan, A.W., Agnew, B.J., Colvin, J.R.
Isolation and characterisation of an anaerobic cellulolytic microorganism
Inst. J. Syst. Bacteriol., 30, 179, 1980

Petersen, G.
Accelerated biogas production from farmyard manure using an anaerobic batch filter digestion
The Stub Report, Presented 2nd Intl. Symp. on Anaerobic Digestion, Travemunde, Germany, Sept. 1981

Richards, K.M.
Anaerobic digestion a source of renewable energy
Poster Presentation, 2nd Intl. Symp. on Anaerobic Digestion, Sept. 1981, Travemunde, Germany, 1981

Rijkens, B.A.
A novel two-step process for the anaerobic digestion of solid waste
Energy Biomass Wastes, 5, 437, 1981

Risser, P.J.
Biomass conversion processes for energy and fuels. (Sofer, S.S. et al eds.) Plenum Press, New York, p.49, 1981

Robbins, J.E., Armold, M.J., Lacher, S.L.
Methane production from cattle waste and delignified straw
Appl. Environ, Microbiol., 38(1), 175, 1979

Robbins, J.E., Armold, M.J., Weiel, J.E.
Anaerobic digestion of cellulose - dairy cattle manure mixtures
Agric. Wastes, 8, 105, 1983

Robertson, A.M., Wolfe, R.S.
Adenosine triphosphate pools in methanobacterium
J. Bacteriol., 102, 43, 1970

Robinson, J.A., Tiedje, J.M.
Competition between sulphate reducing and methanogenic bacteria for H_2 under resting and growing conditions
Arch Microbiol., 137, 26, 1984

Robinson, R.W., Akin, D.E., Nordstedt, R.A., Thomas, A.V., Aldrich, H.C.
Light and electron microscopic examination of methane producing biofilms from anaerobic fixed bed reactors
Appl. Environ. Microbiol., 48(1), 127, 1984

Rozzi, A., Burton, K.W., Hawkes, D.L.
Potentiometric method for the determination of carbon dioxide in biogas
J. Agric. Engng. Res., 28, 505, 1983

Samson, R., LeDuy, A.
Biogas production from anaerobic digestion of Spirulina maxima algal biomass
Biotech. Bioeng., 24, 1919, 1982

Schrass, G., Jewell, W.J.
High rate conversion of soluble organics with a thermophilic anaerobic attached film expanded bed
J.W.P.C.F., 56(3), 226, 1984

Schurz, J.
How to make native lignocellulosic materials accessbile to chemical and microbial attack
Proc. Bioconv. Symp., Delhi, p.37, 1977

Scott, R.I., Williams, T.N., Lloyd, D.E.
Oxygen sensitivity of methanogens in rumen and anaerobic digester populations using mass spectrometry
Biotech. Letts., 5(6), 375, 1983

Shapiro, M., Switzenbaum, M.S.
Initial anaerobic biofilm development
Biotech. Letts., 6(11), 729, 1984

Silvero, C.N., Van, B.T., Pecheco, V.G., Anglo, P.G., Abad, E.J., Alamis, L.A.
Biogas from dried banana peelings
N.S.T.A. Technology Jnl., 8(1), 71, 1980

Smith, P.A., Mah, R.A.
Kinetics of acetate metabolism during sludge digestion
Appl. Microbiol., 14, 368, 1966

Stafford, D.A.
The effects of mixing and volatile fatty acid concentrations on anaerobic digester performance
Biomass, 2, 43, 1982

Stetter, K.O., Gaag, G.
Reduction of molecular sulphur by methanogenic bacteria
Nature, 305, 309, 1983

Stern, M.D., Hoover, W.H.
Methods for determining and factors affecting rumen microbial protein synthesis
J. Animal Sci., 49, 1590, 1979

Stewart, D.
Economics of biogas production from specifically grown plant material
Invermay Res. Centre. Report No.5, 1977

Stuckey, D.C.
Biogas in developing countries
Proc. 3rd. Intl. Symp. on Anaerobic Digestion, Boston, Mass., Aug. 1983, p.253, 1983

Szendrey, L.M.
Start-up and operation of the Bacardi Corporation anaerobic filter
Proc. 3rd. Intl. Symp. on Anaerobic Digestion, Boston, Mass., Aug. 1983, p.365, 1983

Thomm, M., Altenbuchner, J., Stetter, K.O.
Evidence for a plasmid in a methanogenic bacterium
J. Bacteriol., 153(2), 1060, 1983

van Velsen, A.F.M.
Anaerobic digestion of piggery waste. 1. The influence of detention time and manure concentration
Neth. J. Agric. Sci., 25, 151, 1977

van Velsen, A.F.M.
Adaptation of methanogenic sludges to high ammonia concentrations
Wat. Res., 13, 995, 1979

Verrier, D., Roy F., Florentz, M.
Two-stage anaerobic digestion of solid vegetable waste
Poster Presentation, 3rd Intl. Symp. on Anaerobic Digestion, Boston, Mass., Aug 1983

Verstraete, W.
Biomethanation of wastes : perspective and potential
Proc. Intl. Conf. Bisotech.'83, p.725, 1983

Vogels, G.D., Visser, C.M.
Interconnection of methanogenic and acidogenic pathways
F.E.M.S. Microbiol. Letts, 20(3), 291, 1983

Wallace, R.J., West, A.A.
Adenosine 5' triphosphate and adenylate energy charge in sheep digesta
J. Agric. Sci. (Camb.), 98, 523, 1982

Walter, D.K.
Anaerobic digestion of municiple solid waste to produced methane
Energy Biomass Wastes, 7, 705, 1983

Wang, H.H., Liu, C.H., Lu, C.C.
Energy ratio of agricultural waste utilisation with appropriate and intermediate technology
Proc. Intl, Symp., on Biogas, Microalgea and Livestock wastes, p.191, 1981

Webb, A.R.
Factors influencing methane production during the anaerobic digestion of poultry wastes.
Ph.D. Thesis, CNAA, 1984

Wiegnant, W.M., Zeeman, G.
Influence of ammonia on the thermpophilic digestion of cairy cow slurry
Poster Presentation, 3rd Intl. Symp. on Anaerobic Digestion, Boston, Mass., Aug. 1983

Wise, D.L. Wentworth, R.L., Augenstein, D.C., Cooney, C.L.
Multistage digestion of municiple solid waste for fuel gas
Resour. Recor. Conserv., 32, 41, 1978

Woese, C.R., Magnum, L.J., Fox, G.E.
Archaebacteria
J. Mol. Evol., 11, 245, 1978

Wolfe, R.S.
Biochemistry of methanogens
Experienta, 38(2), 198, 1982

Wolfe, R.S.
Methanogens : a surprising microbiological group
Antonie van Leeuwenhoek, 45, 353, 1979,

Wolin, M.J., Miller, T.L
Interspecies hydrogen transfer : 15 years later
A.S.M. News, 48(12), 561, 1982

Wolstrupp, J., Jensen, K.
A.T.P. in the bovine rumen during maximum nutrient supply and starvation
J. Appl. Bacteriol., 41, 243, 1976

Wong-Chong, G.M.
Dry anaerobic digestion. In : Agriculture and Waste Management (Jewell, W.J., ed.) Ann Arbor Science, p.361, 1975

Wood, T.M.
Cellulose and cellulolysis
World Rev., Nutr., Diet, 12, 227 1970

Zehnder, A.J.B.
Ecology of methane formation
Wat. Poll. Microbiol., 2, 349, 1978

Zeikus, J.G.
Fate of lignin and related aromatic residues in anaerobic environments. In: Lignin Biodegradation
(Kirk, T.K. et al, eds) C.R.C. Press, Boca Raton, 1980a

Zeikus, J.G.
Microbial population in digesters. In: Anaerobic digestion
(Stafford, D.A. et al., eds) Applied Science, London, p.61, 1980b

Zeikus, J.G., Wolfe, R.S.
Methanobacterium thermautotrophicum, an anaerobic autotrophic extreme thermophile
J. Bacteriol., 109(2), 707, 1972

Zimmerman, R.E., Lytwynshyn, G.R., Wilkey, M.L.
Landfill gas recovery. A technology status report
Argonne National Laboratory. Report No. ANL/CNSY TM121, 1983